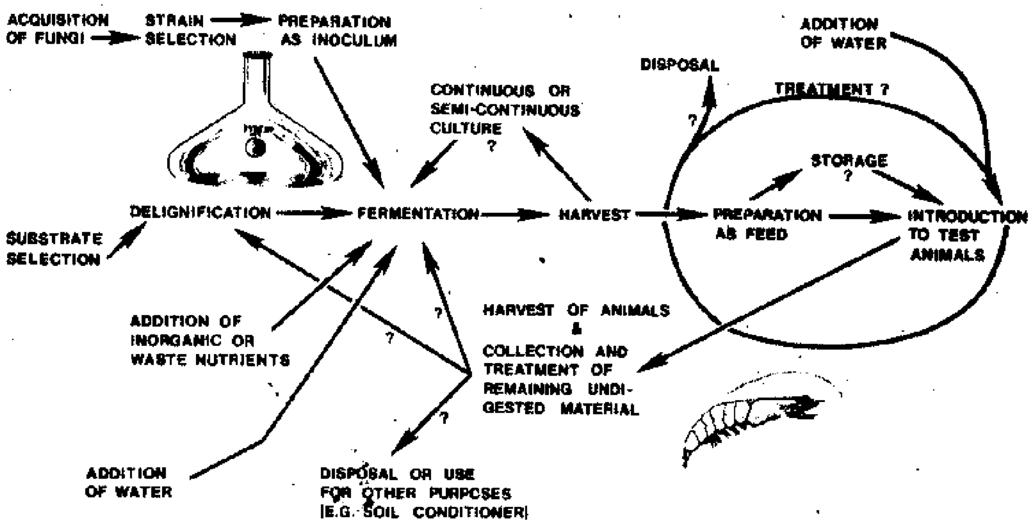
Preliminary Experimentation in the Development Of Natural Food Analogues For Culture of Detritivorous Shrimp



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PRELIMINARY EXPERIMENTATION IN THE DEVELOPMENT OF NATURAL FOOD ANALOGUES FOR CULTURE OF DETRITIVOROUS SHRIMP

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ABSTRACT

The fungi are the primary mediators of the microbial degradation of vascular plant debris to particulate detritus in aquatic and estuarine ecosystems. Several estuarine animals of commercial food value including penaeid shrimp are known to be nutritionally dependent, at least in part, on the microbial-detrital complexes resulting from degradation of vascular plant debris. Thus, fungi form a natural part of the diets of these animals.

Recent extensive research has demonstrated that the fungi are very attractive as agents of the manufacture of nutritious microbial protein on an industrial scale. The cost of feed is one of the factors which yet prevents the commercial profitability of aquaculture of penaeid shrimp. If, as seems reasonable, agricultural by-products can be converted inexpensively into fungal materials simulating the naturally eaten microbial-detrital complexes, effective low-priced feeds may be developed. This technical bulletin describes a 3-year preliminary effort at testing of this hypothesis.

Fungal fermentations of agricultural by-products were conducted and attempts were made to optimize fungal protein production. Resultant artificial detrital feeds were tested on a proto-commercial scale in outgrowth of penaeid shrimp. Yields of shrimp ranged from very poor to encouragingly high, considering conditions imposed. Screening was begun of fungal capability to degrade the least expensive types of agricultural by-products, when these were modified to reduce lignin-associated refractoriness. Perhaps the most significant of the conclusions to be reached is that successful development of artificial detrital feeds is not likely to be realized without a major commitment of time, expertise, and money. Suggestions are presented for future research directions, and range of applicability is briefly discussed.

PRELIMINARY EXPERIMENTATION IN THE DEVELOPMENT OF NATURAL FOOD ANALOGUES FOR CULTURE OF DETRITIVOROUS SHRIMP

INTRODUCTION

1. Studies of formulation of artificial feed for shrimp

ne unrealized potential of shrimp mariculture as a viable commercial industry nt from the value of the current shrimp fisheries industry (Roedel, 1973). The [illegible] is e[illegible] cost of the feed necessary to bring about the growth of satisfactory, marketable crops of penaeid and Macrobrachium shrimp is one of the major obstacles to the emergence of a profit-producing shrimp mariculture industry (Anderson and Tabb, 1971; Idyll, 1973; Shang, 1974; Webber, 1973). The profitable shrimp culture industry of Japan is dependent upon the high market price of shrimp in that country (Bardach et al., 1972), but even in Japan, feed costs are presenting problems (Hanks, 1973), [illegible] research into artificial feed preparations is underway there (Deshimaru and Shi[illegible] 1972). In the United States, where shrimp prices are considerably lower, profita[illegible] has eluded those business organizations which have sought to achieve it in sh[illegible] farming, and research into formulation of inexpensive yet effective feeds is o[illegible] the steps being taken to overcome this problem (Neal, 1973).

Results of feed formulation research have varied among researchers, [illegible] conclusions are difficult to draw. There is disagreement regarding the optimal le[illegible] of major and minor components; for example, Deshimaru and Shigeno (1972) indic[illegible] that artificial feeds for Penaeus japonicus should contain at least 60% protei[illegible] more, while Andrews et al. (1972) concluded that 28-32% protein produces the [illegible] growth of penaeids. Shigeno et al. (1972) found a positive correlation of pro[illegible] content (between 60% and 80%) with feed efficiency, and Balazs et al. (1973) belie[illegible] that their data demonstrated that the optimal protein level might be greater [illegible] 40%, but Shewbart et al. (1973) found that the optimal protein content lay betw[illegible]

22.5% and 30.5%. Zein-Eldin and McGaffey (1976) found what is probably part of the reason for these apparent conflicts; their work showed that percent protein of over 50 gave the best growth results when one protein source was used, but when a different source was used, 32% protein produced better growth. They suggested that protein quality affected the relationship between shrimp growth and percent protein in the diet. Other feed component requirement levels about which there is seeming disagreement in the literature include aliphatic lipids and their fatty acids, carbohydrates, sterols, glucosamine or chitin, and binding and attracting agents. The clearest conclusion to be drawn from examination of the above and other recent studies of shrimp ration requirements (Andrews and Sick, 1973; Balazs et al., 1975; Deshimaru and Kuroki, 1974a-c, 1975; Forster, 1971, 1973; Forster and Gabbott, 1971; Hysmith et al., 1973; Kanazawa et al., 1970, 1971; Kitabayashi et al., 1971a-c; Meyers and Zein-Eldin, 1973; Shudo et al., 1971; Sick and Andrews, 1974; Sick and Harris, 1975; Venkataramiah et al., 1975; Zein-Eldin and Meyers, 1974) is that the interacting effects of protein, lipid, carbohydrate, and other components in shrimp diets make the optimal formulation of artificial feeds very difficult.

Taub (1973) has noted that one means of finding satisfactory diets for aquacultured animals is to simulate their natural diets. The natural diets of penaeid and Macrobrachium shrimp may include sizable quantities of the plant detritus produced in the estuaries and bordering waters which these shrimp inhabit (Bardach et al., 1972; Condrey et al., 1972; Cook and Lindner, 1970; Costello and Allen, 1970; Dall, 1967; Ling, 1969; Odum and Heald, 1972). One approach to determination of an adequate diet for these animals is to simulate this natural plant detritus.

2. The formation of plant detritus in estuaries

[illegible] (1972) define "biodegradation" as "the useful breakdown or [illegible]
investigated [illegible] conversion of materials to a higher nutritive value." [illegible]
[illegible]rrence of this process in a study of the energy flow through a South [illegible] the natural o[illegible]

Florida estuary, finding that the food web of this shrimp nursery ground (Kutkuhn, 1966) was based largely on the production of large quantities of debris by mangroves. This plant debris was found to undergo microbial biodegradation to detrital particles which were higher in protein content than the original material (Fell et al., in press; Newell, 1975). These microbial-detrital complexes served as a food source for meiofaunal and larger detritivores of the estuary. The pattern of dependence of food webs upon secondary microbial production applies generally to aquatic ecosystems with relatively large ratios of shoreline length to water-surface area (Mathews and Kowalczewski, 1969).

Recent studies have demonstrated that the phenomenon of protein enrichment of low-protein submerged plant debris is related most directly to the activity of fungi (Bärlocher and Kendrick, 1974; Gessner et al., 1972; Kaushik and Hynes, 1971; May, 1974; Triska, 1970; Willoughby and Redhead, 1973). Fatty acid enrichment due to fungal colonization of plant debris has also been indicated (Schultz and Quinn, 1973). It is reasonable to conclude, therefore, that shrimp in estuarine habitats derive a portion of their nutrition from the fungi which they ingest in microbial-detrital complexes.

3. Fungi as producers of SCP

Recognition of the fact that conventional methods of production of protein for human consumption are not meeting the present-day need, and that this situation will continue to worsen, has led to a great deal of research into development of more efficient means of production of high protein material (A. Jones, 1974). Much of this research has dealt with the production of microbial or single-cell protein (SCP), and it is reviewed by Mateles and Tannenbaum (1968), Kihlberg (1972), and Porter (1974a, b). Substrate sources for the fermentation processes involved in the production of SCP have included high-starch-content agricultural products (e.g., cassava) and a wide variety of agricultural and food-industry wastes.

Although filamentous fungi are not single-cell organisms, they are generally included among agents of SCP production. Reviews of the use of fungi as producers of protein, food and feed are given by Cooke (1973), Gray (1970), Hesseltine (1968), Litchfield (1968), Solomons (1975), Thatcher (1954), and Worgan (1968, 1973). The principal advantages in the use of filamentous fungi in SCP processes are economic. In submerged culture fermentations, because of their growth form, these fungi can be more easily harvested, by basket centrifugation or other coarse filtration methods, and the resultant product is more easily processed into textured food or feedstuff than those of single-cell organisms (Spicer, 1971, 1973; Worgan, 1973). Perhaps the greatest economic advantage of filamentous fungi lies in their amenability to a less conventional (in the West) type of fermentation, viz. solid state fermentations. This point will be further explored below (Section 15).

Recent reports of filamentous fungal protein production research include (substrate in parentheses): Barnes et al. (1972), Biodeterioration Information Centre of the University of Aston in Birmingham, England (waste paper); Bednarski et al. (1970, 1971), Agricultural University of Olsztyn, Poland (milk whey); Brook et al. (1969), Tropical Products Institute, England (cassava); Chahal and Gray (1970) and Chahal et al. (1972), Punjab Agricultural University, India (wood pulp and rice); Church et al. (1972), North Star Research and Development Institute, Minnesota (corn-canning waste and soybean whey); Imrie (1973, 1975), Sekeri-Pataryas et al. (1973) and Christias et al. (1975), Tate and Lyle Research Center, England, Greece, and Belize, Central America (carob bean extract and citrus waste); Poole and Smith (1973), University of Aberdeen, Scotland (straw); Reade et al. (1974), University of Guelph, Canada (cassava); Rogers and Spino (1973), National Environmental Research Center, Ohio (lignocellulosic wastes); Spicer (1971, 1973), Solomons (1973, 1975), Solomons and Scammell (1974a, b), and Anderson et al. (1975), Lord Rank Research Centre, England (several carbohydrates); Thanh and Simard (1973), Laval University, Canada (domestic sewage); Torev (1973), Bulgaria, where industrial-scale production of polypore mycelium for use as human food ("sausages") is now operating (according to Von Hofsten, 1975); Updegraff (1971), University of Denver, Colorado (waste paper); Von Hofsten and Von Hofsten (1974), University of Uppsala, Sweden (cereals and cereal by-products); Wiener and Rhodes (1974) and Griffin et al. (1974), Northern Regional Research Laboratory, Illinois (feedlot wastes); Worgan (1973; and J. Sci. Fd. Agric., in preparation), National College of Food Technology of the University of Reading, England (food processing wastes). Even waste plastics are being used, with some success, as substrates in the production of filamentous fungal protein (Brown et al., 1974). Perhaps the most concerted effort has been that of the Lord Rank Research Centre, which has progressed to the point of attempting satisfaction of international food-safety regulations with its fungal protein product, having proven the nutritive value and non-toxicity of their fungal protein product for rats, chickens, pigs, calves, and baboons (Duthie, 1975).

4. Use of fungal biomass as feed

Many of the above researchers have tested their experimental protein products as feed for laboratory animals (rats, mice, chickens) and positive results have been obtained with regard to growth potential. For example, Reade et al. (1974) reported that a protein efficiency ratio of 2.3 was achieved when their Aspergillus product was fed to rats. Solomons (1973) reported that true protein digestibility (by rats) of one of his filamentous fungal products was virtually 100%. This same product gave growth results (and non-toxicity) equal to that of a casein diet with baboons (Duthie, 1975). Church et al. (1972) reported equal growth rates among rats fed a casein-protein diet and rats fed a fungal (Trichoderma viride) - protein diet. Evidence for the utility of fungi as a feed source has also come as an outgrowth of research intended to pinpoint the causes of feed quality reduction or toxicity by moulding; positive effects on growth were discovered in some farm animals (chickens, sheep, pigs) and laboratory animals (mice, rats) when specific moulding conditions (physical and chemical fermentation conditions and fungal strains) were used (Chah et al., 1973; Fritz et al., 1973; Marasas and Smalley, 1972; Richardson et al., 1967; Sharda et al., 1971; Thomke,

... 1973b, b). Dabbah et al. (1965) and Pector et al. (1968) achieved positive growth

ice and rats with the mycelial by-product of penicillin production used as
t for soybean and casein fractions of diets. See Anon. (1971) for a
of further reports of this kind.

rowth-promoting qualities of fungal feeds are not surprising if the
itritive quality of fungal mycelium is taken into account. The Reade and
is products mentioned in the foregoing paragraph contained 48% (crude)
50% (true, = total amino acids) protein respectively. Although most
roteins are low in methionine content relative to the requirements of the
which they might be fed, the range of methionine contents of filamentous
ies above that of the FAO minimum requirement level for humans
t al., 1975; Rhodes et al., 1961). Nonetheless, as Church et al. (1972) and
1972) point out, methionine addition is commercially feasible since this
is now inexpensive and easily obtainable. Furthermore, Kanazawa et al.
Sick and Anderson (1974) have found that soybean meal, which is
low in methionine content," served better as a protein source for penaeid
some animal proteins. Other than the sulfhydryl amino acids, fungal
general contain nutritionally satisfactory levels of essential amino acids,
a wide range of specific amino acid contents. This is true even among
he same genus (Rhodes et al., 1961). Therefore, if one were to search for a
rce having a given amino acid balance, it is likely that a species or strain
ould be found possessing that quality.

ps most notably important in this regard is the tractability of the protein
fungi. Levi and Cowling (1969), Litchfield et al. (1963), Merrill and Cowling

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(1969) demonstrated that fungi were able to adapt their nitrogen and protein contents to the C/N ratios of their substrates. Their reports, along with that of Graham (1971), show that the increase in nitrogen reflects an increase in protein content, not in glucosamine (or chitin) or cellular ammonia. Alteration of other physical and chemical conditions of culture can also be used to adjust protein and amino acid content, including methionine content (Chebotarev and Zemlyanukhin, 1973; Florentino and Broquist, 1974; Graham, 1971; Pinto, 1963; Solomons, 1973; Tkachenko et al., 1972; Verona et al., 1973). In addition, the chitin content of cell walls of higher filamentous fungi varies greatly from species to species, and the chitin percentage of mycelial biomass varies greatly with age of culture (Blumenthal and Roseman, 1957). The wide range of contents of nutritively valuable components of fungal mycelium together with the manageability of the relative amounts of these components in a given species, validate Gray's (1970) "optimistic view that if one searches far enough and widely enough, he can often find a fungus to perform the particular synthesis {or serve the purpose} in which he is interested."

Three studies of the use of filamentous fungi which relate directly to the potential use of fungal feeds for shrimp are those of Bärlocher and Kendrick (1973, 1975), Joshi (1974), and Nikolei (1961). These are reports of the use of fungal tissue in the culture of arthropods which feed naturally on dead, fungal-decayed plant material. The Bärlocher and Kendrick studies were intended as sequels to the studies of Kaushik and Hynes (1971) and Triska (1970) (Section 2) who had shown that the fungi were responsible for the major portion of the protein increase in decaying plant debris, and that detritus-feeding crustaceans definitely preferred fungal-invaded material over sterile material. Bärlocher and Kendrick fed leaves with a minimal microbial population, leaves richly colonized by microbes, and fungal mycelia (10 species), separately to cultures of a detritivorous gammarid amphipod. Some of the fungi proved to be excellent growth-promoting food, better than the leaf material, while other fungi produced little or no growth.

Joshi fed fungal meals of differing types to a mycophagous thysanopteran; he found that the length of the larval period and adult fecundity were markedly affected by the species of fungus used in preparation of the meals.

Nikolei found similar results in his search for suitable fungal tissue to serve as a culture medium for three species of cecidomyiid flies. He tested 67 species of fungi and seven strains of Trichoderma viride. Fifty of the species were unsuitable for culture of the fly larvae and of the seventeen which were suitable, only four were suitable for all three fly species. There was considerable variability among the T. viride strains in nutritive capacity which ranged from highly positive to distinctly negative. Also, as Bärlocher and Kendrick had found or suspected, culture conditions and age of the fungi had marked effects on their growth-producing potential. Again, Gray's (1970) "if one searches far enough ..." viewpoint seems to be borne out.

Although the present authors know of no published reports of the feeding of fungal mycelia to postlarval or juvenile commercially valuable shrimp, the following reports indicate its plausibility. R. R. Jones (1974) has demonstrated that, as in the case of other detritivorous crustacea (Fenchel, 1972; Hargrave, 1970), natural detrital materials are nutritively utilized by young juveniles of Penaeus aztecus with high protein assimilation efficiency. Even the cell walls of the fungi probably serve as sources of shrimp nutrition. The cell walls of the higher filamentous fungi are chitinous (LéJohn, 1971), and Hood and Meyers (1974; and unpublished communication) have shown that penaeid shrimp have, in addition to a hepatopancreal chitinase, a bacterial gut flora which is 85% chitinolytic and which increases in size with addition of chitin to the diet of the shrimp. Minami et al. (1972) have shown that the size of the chitinolytic bacterial population of fish guts is positively correlated with their efficiency of utilization of petro-yeast. The requirement of penaeids for a chitin component in their diets has been examined, but disagreement concerning the nature of this requirement exists (compare Deshimaru and Kuroki, 1974b; Kanazawa et al., 1970; Kitabayashi et al., 1971a).

The following reports are also of interest in this connection. Ewald (1965) used marine yeast (a Rhodotorula species) as a component of the food which he used in the early successful work with raising of larval Penaeus duorarum. Forster (1973) found that the nitrogen in a yeast-protein component of a palaemonid shrimp ration was assimilated at 95% efficiency, and Andrews and Sick (1973) found that yeast-protein labeled with carbon 14 was incorporated at a high rate into penaeid shrimp muscle tissue. Meyers et al., (1970) found that young freshwater crawfish grew "encouragingly" when fed a diet consisting of 39% spray-dried yeast. In a very important personal communication, Dr. E. B. G. Jones (Portsmouth Polytechnic, England) reports that he and his colleagues have found that commercially culturable shrimp will ingest fungal mycelia, and that "reasonably good" yields have resulted.

MATERIALS, METHODS, AND RESULTS

5. History of an artificial detrital feed project

In an attempt to reduce the cost of feed used in shrimp mariculture, Drs. C. W. Caillouet and D. C. Tabb of the University of Miami Rosenstiel School of Marine and Atmospheric Science (RSMAS) initiated research into the use of artificial detrital [illegible] project was a seemingly natural seque[illegible] [illegible] dum et al. (1973), also done at RSMAS, which [illegible] to those of Odum and Heald (1972) an[illegible] al material in the mangrove nursery grounds [illegible] dealt with the natural formation of de[illegible] [illegible] of pink shrimp. Caillouet and Tabb [illegible]

As an adjunct project, we (unpublished data) conducted a microbiological and gross biochemical survey of the artificial detrital materials from the shrimp tanks over a period of four weeks. Litter-bag sampling methods similar to those of Newell (1975) were used. One major deviation from those methods was the added emphasis placed on results from incubation of pieces of the materials in dishes of sterile seawater (15 o/oo) with antibiotics (penicillin and streptomycin, 0.05%).

The survey showed that, although distinct fungal florae had inhabited the different detrital material (Table 1)*, nitrogen enrichment of the materials did not take place. During the four weeks of sampling fungal colonization increased as bacterial colonization decreased, and the materials lost about half of their original nitrogen content. The biodegradation phenomena which Caillouet and Tabb had hoped for did not take place in their tanks. The major reasons for this were probably: (1) that significant increase in nitrogen content of lignocellulosic materials due to fungal biodegradation requires more time than was allowed; (2) that the conditions requisite for permitting the fungal biodegradatory process were not supplied; (3) little or no natural inoculum was present (note {Table 1} the marked differences in fungal occurrence patterns in the tanks as opposed to the adjacent mangrove pond); and (4) unavailability of macronutrients and buildup of fungal growth inhibitors may well have been brought about as a consequence of the standing condition of the water of the tanks. Yield of shrimp in the experiment, rather than being related to extent of

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* The following are points of note from Table 1. The bagasse had the heaviest fungal inoculum at the outset. Further colonization was most extensive on bagasse in the tanks, but the difference in extent of colonization was less marked in the mangrove channel. Some fungi present initially on the substrates were not reduced in frequency of occurrence by submergence (e.g., _Trichoderma viride_, _Syncephalastrum racemosum_) while others were eliminated (_Chaetomium_ sp.), or reduced (_Alternaria_ sp.). Some fungi colonized more extensively in the tanks (_Stachybotrys_ sp.), while others were equally frequent in the mangrove channel (_Trichoderma viride_, _Zythia_ sp. on straw). The obligate marine fungi (_Lulworthia_ sp., _Zalerion varium_, _Thraustochytrium pachydermum_) colonized only in the mangrove channel.

Table 1. Frequency (%) of occurrence for the prevalent fungi which colonized materials used as artificial detrital feeds in mariculture of penaeid shrimp.

	WHEAT BRAN			WHEAT STRAW			BAGASSE		
	PB[a]	TK	MC	PB	TK	MC	PB	TK	MC
no fungi	100	71	4	89	34	13	44	3	2
diatoms (heterotrophy)[b]	0	28	0	0	20	0	0	0	0
sterile mycelium	0	25	25	0	33	27	6	3	2
Hyphomycetes									
Alternaria sp.	0	0	0	11	3	0	0	3	0
Ascotricha chartarum	0	0	0	0	0	0	0	2	0
Aspergillus spp.	0	0	0	0	0	0	0	8	11
Cephalosporium spp.	0	0	15	0	0	12	6	57	65
Cirrenalia pseudomacrocephala	0	0	4	0	0	5	0	0	6
Cladosporium sp.	0	2	9	0	0	0	0	0	8
Colletotrichum sp.	0	0	4	0	0	0	0	0	0
Culcitalna achraspora	0	0	0	0	0	3	0	0	0
Dendryphiella arenaria	0	0	0	0	0	0	0	0	8
Fusarium sp.	0	0	0	0	0	0	0	7	0

Table 1 (continued)

	WHEAT BRAN			WHEAT STRAW			BAGASSE		
	PB[a]	TK	MC	PB	TK	MC	PB	TK	MC
Paecilomyces sp.	0	0	4	0	0	0	0	6	0
Penicillium spp.	0	0	7	0	0	0	0	21	29
Stachybotrys sp.	0	0	0	0	0	3	0	42	13
Trichoderma viride	0	0	0	0	0	0	6	23	22
Zalerion varium	0	0	0	0	0	7	0	0	5
Coelomycetes									
Zythia spp.	0	0	11	0	12	12	6	17	5
Ascomycetes									
Chaetomium sp.	0	0	0	0	0	0	22	0	0
Lulworthia sp.	0	0	13	0	0	5	0	0	5
Zygomycetes									
Syncephalastrum racemosum	0	0	0	0	0	0	6	10	5
Chytridiaceous fungi									
Thraustochytrium pachydermum[c]	–	0	33	–	0	27	–	0	61
Phlyctochytrium mangrovii[c]	–	0	10	–	0	11	–	0	16

Table 1 (continued)

	WHEAT BRAN			WHEAT STRAW			BAGASSE		
	PB[a]	TK	MC	PB	TK	MC	PB	TK	MC
bacteria[d]	-	0.10	0.16	-	< 0.01	0.09	-	0.04	0.05
N%[e]	2.6	1.3	1.8	0.4	0.1	0.1	0.4	0.2	0.4
number of observations	18	126	55	18	126	63	18	126	60

[a] PB=sampling prior to placement in litter bags; TK=sampled periodically after placing in litter bags in shrimp tanks; MC=sampling after placing in litter bags in a mangrove lined estuarine channel adjacent to the shrimp tanks.

[b] Includes only those observations made on pieces of the material which were washed, surface-sterilized, rinsed, and plated on cornmeal agar.

[c] Includes only those observations made on pieces of the material which were washed and incubated in dishes of 15 $^{o}/oo$ seawater with 0.05 % penicillin and streptomycin, and baited with boiled blades of drop-seed grass (*Sporobolus virginicus*).

[d] The figures given are the ratios of final number of viable elements to initial numbers of viable elements per piece of material (determined by membrane (0.45 μm) filtration of dilutions of the wash water, and plating of the membranes on a glucose-peptone-yeast extract agar).

[e] Nitrogen percentage at final sampling. Comparison of the PB samples with TK and MC samples show change in N% from original.

biodegradation, was positively correlated with the original nitrogen content of the materials; shrimp fed wheat bran (N% = 2.7) were on the average over twice as large as those fed the nearly purely lignocellulosic straw and bagasse (N% = 0.4).

6. Culture-screening on wheat bran and bagasse

Background.

The next logical step in the development of artificial detrital feeds was an acceleration of the natural biodegradation process. This we hoped to accomplish by moving the process from the shrimp tanks into separate containers in which the biodegradation could be conducted as controlled, submerged-culture, fungal fermentations. We planned our fermentation experimentation for use of diluted (15 o/oo) seawater because: (1) penaeid culture is most economically accomplished as a coastal process; (2) fresh water is one of the earth's threatened resources; (3) Gray et al. (1963) had shown that use of seawater in fungal fermentations could produce enhancement of protein production; (4) the estuarine-adapted fungi form a portion of the natural shrimp diet (Section 2); and (5) both terrestrial and obligately marine fungi in general can proliferate in partial seawater (Jones et al., 1971).

Materials and methods.

We began by conducting a culture screen of 29 isolates of fungi and 5 mixed cultures of fungi for their ability to increase the proteinaceous material in wheat bran and bagasse. A diversity of fungal species was used (Table 2), including fungi restricted to marine-estuarine environments (e.g. Lulworthia spp.), fungi isolated from the plant materials during the Caillouet-Tabb shrimp-feeding experiment (e.g. Stachybotrys sp.), and other fungi recently isolated from naturally decaying plant materials in local estuaries, some of which had been indicated as potentially efficient protein producers (e.g. Trichoderma viride by Church et al., 1972).

Shake flask fermentations were performed as prescribed by Solomons (1969) and by adapting the methods of Sguros et al. (1962). A cylinder (2 cm^2 diam.) was removed (sterile #14 cork borer) from the growing edge of a colony of each of the test fungi on cornmeal (Difco) agar (CMA) made with 15 o/oo seawater. The cylinder was placed in the bottom of a presterilized Monel Metal Waring microblender in 25 ml of sterile 15 o/oo seawater, and homogenized for 60 seconds at low speed. One ml of the homogenate was added, in a 125 ml DeLong flask, to 25 ml of the inoculum-growth medium: glucose - 1%, NH_4NO_3 - 0.24%, KH_2PO_4 - 0.006%, yeast extract (Difco) - 0.01%, cellulose (powdered Whatman filter paper) - 0.01%, pectin - 0.01%, in 15 o/oo seawater. The flasks were capped with Belco metal closures, sealed around the lower edges of the caps with clear tape (to inhibit contamination), and incubated at 28°C on a temperature-controlled reciprocating shaker (70 cycles/min. through 8 cm distance). These inoculum-growth flasks were harvested at approximately the early stationary phase (3 to 9 days). As was the case with Wang et al. (1974), only subjective determinations of growth phase were made due to the difficulties inherent in determining quantities of mycelia mixed with solid substrate residues (Calam, 1969b). The sieve-separation method of Borzani et al. (1972) was not attempted. The entire content of each flask was homogenized for 60 seconds, and 2 ml of homogenate was added, in each of two 125 ml final culture-screen flasks, to 25 ml of the following media: one of 5% ground bagasse (40-mesh) and one of 5% ground wheat bran, with the remainder of the media the same as that of the inoculum-growth medium, with the omission of the cellulose and pectin. These final flasks were incubated as were the inoculum-growth flasks.

Ideally, the inocula would have been washed and their dry weights standardized. This was avoided for the following reasons: impracticability in view of limited time and personnel; severe increase of contamination risk; ineffectiveness of this method of standardization of inoculum potential. Our work (Table 5) has shown that equal times of homogenization produce large variations among different fungi in numbers of viable mycelial elements per unit dry weight. This can have a marked effect on inoculum potential (e.g. Graham, 1971). The mycelium exhaustion steps of the inoculum preparation of Sguros et al. (1962) were not used in the present experiment, as this would certainly not be a part of production-scale fermentation processes, in which optimization of vigor of inoculum is desired.

The work of Sguros et al. (1962) and Ward and Colotelo (1960) on systematically divergent fungi both showed that amounts of inoculum of dry weight greater than 2 mg (into 25 ml of medium) do not have marked effects on final yield of mycelium. We calculated from the papers of Sguros et al. (listed in Sguros, 1973) and Meyers (reviewed in Meyers, 1971) that 2 ml of homogenate produced as above would contain at least 2 mg of mycelium. Upon inoculation of each culture screen flask, a second, control 2 ml of homogenate was vacuum-filtered on a tared glass filter pad (Whatman GF/A), washed with 50 ml deionized water, and dried at 55°C. The range of inoculum dry weights was 2.3 mg (Stachybotrys kampalensis SC76) to 11.7 mg (Pestalotia sp. SC 38). In order to determine the effect of unequal inocula, a regression was performed, of final nitrogen percentage of fermented wheat bran on inoculum weight. Significance was detected ($p = 0.001$) with a slope of $- 0.11$ and the coefficient of determination (r^2) = 0.25; those fungi which produced heavier inocula tended to produce lower final nitrogen percents in fermented wheat bran, and 25% of the variation in percent nitrogen was due to inoculum size. This was probably primarily a function of the positive correlation between inoculum size and growth rate: the fastest-growing forms produced larger inocula, and were likely to be harvested at a point in their growth cycles beyond early stationary phase.

Harvesting of fermented product was done at approximately early stationary growth phase by coarse (42 μm), double-cloth filtration, with tap water washing until soluble nitrogen was removed (2 equal volumes of tap water, with intervening through resuspensions). The products were then dried at 55°C and ground through 60-mesh Wiley mill screen. Nitrogen content was analyzed using a Perkin-Elmer Model 240

Elemental Analyzer, and protein figures given are for crude protein (N x 6.25).

Further screens of fungal isolates for their capacity to convert wheat bran into fungal protein were conducted using a second standard set of fermentation conditions raising the total number of isolates screened with wheat bran to 52. The medium used in the second set of wheat bran screens was: wheat bran, unground - 5%, NH_4NO_3 - 0.86%, KH_2PO_4 - 0.09%, yeast extract - 0.01%, in 15 o/oo seawater. Inoculation of 50 ml of medium in 250 ml Ehrlenmeyer flasks was by addition of 2 ml of CMA culture homogenate as in the first wheat bran screen (inoculum growth flasks were not used). Incubation, harvesting, and analysis were the same as in the first wheat bran screen.

Inorganic nitrogen and phosphorus content of the medium for the second screen were increased in order that maximal biomass and crude protein production would be permitted (calculated on the basis of approximate theoretical maximal conversion of wheat bran and maximal potential content of nitrogen and phosphorus in mycelia; several references were used in this calculation, principally Burnett, 1968; Cochrane, 1958; Gray, 1970; and Litchfield, 1968.)

Results.

Results of the culture screens are given in Tables 2 and 3 and Figure 1. The figures are for the solid end products of the fermentations, fermented plant particles plus fungal mycelium. Although only one of the wheat bran products was lower in percent protein than the original material, the range of crude protein increments was from -46.5% (Syncephalastrum racemosum, Table 2) to +50.6% (Myrothecium sp., Table 3). The medium used in the first bran culture screen was 3.6 times lower in inorganic nitrogen than the medium used in the later culture screen. Probably as a partial consequence, there were more losses in crude protein observed in the first wheat bran screen than in the second (Fig. 1). Bagasse conversions (Table 2) were much poorer than with wheat bran. Although there were some high increments in

Table 2. Results of the first culture screening intended to detect strains of fungi optimally able to convert wheat bran and bagasse into mycelial biomass.[a]

FUNGAL STRAINS[b]	WHEAT BRAN		BAGASSE	
	CP%[c]	ΔCP%[d]	CP%	ΔCP%
Lulworthia sp. SC73	28.9	+16.9	2.9	-12.8
SC73 & SC51	27.9	+15.4	2.9	-15.3
Leptosphaeria maritima RZ312	25.8	+14.5	2.7	-15.3
Leptosphaeria discors SC71	25.4	+ 3.3	2.9	-13.0
Zalerion varium SC21	24.1	+ 2.9	2.9	-12.8
Zalerion varium RZ264	26.4	+ 2.2	3.1	- 7.9
Trichoderma viride SC51	24.8	+ 2.1	3.3	- 5.2
Drechslera hawaiiensis RZ17	24.5	+ 0.1	--[f]	--
Lulworthia medusa v biscaynia RZ 281	26.1	- 0.1	5.3	+53.7
Lulworthia grandispora RZ389	24.4	- 1.5	--	--
Stachybotrys kampalensis SC76	23.9	- 2.1	--	--
Zalerion varium RZ394	23.8	- 3.5	3.3	- 0.5
Culcitalna achraspora SC24	23.0	- 4.8	--	--
Corollospora maritima SC67	23.4	- 6.0	4.0	+14.5
Stachybotrys sp. SC52	25.3	- 7.1	4.6	+22.2
Culcitalna achraspora RZ387	22.4	- 8.9	3.8	+15.5
Leptosphaeria albopunctata SC70	22.1	-12.2	3.6	+ 4.2
Cirrenalia pseudomacrocephala RZ280	22.8	-12.4	2.8	-17.0
SC47 & SC70	23.0	-13.8	3.5	+ 2.2
Pestalotia sp. SC38	20.4	-14.8	4.0	+20.7
Zalerion varium RZ393	21.7	-15.8	3.2	- 6.2
Clavariopsis bulbosa SC66	22.4	-17.1	5.5	+45.0
Corollospora maritima SC68	22.6	-17.2	6.3	+68.9
Ascotricha chartarum SC44	20.0	-18.4	2.5	-26.1
SC73 & SC62	21.8	-19.3	2.8	-19.0
Hyalostachybotrys sp. SC74	18.7	-24.9	4.7	+40.6
Nia vibrissa SC72	16.9	-25.6	3.6	+ 8.6
SC47 & RZ387	18.9	-28.9	2.6	-21.4
Hyalostachybotrys sp. SC62	19.9	-29.4	3.5	- 1.0

Table 2.—(continued)

FUNGAL STRAINS[b]	WHEAT BRAN		BAGASSE	
	CP%[c]	ΔCP%[d]	CP%	ΔCP%
SC47 & RZ393	18.4	-32.5	4.4	+28.8
Robillarda sp. SC75	17.3	-33.5	2.1	-34.7
SC47 & SC73	17.3	-37.4	3.3	- 4.7
Syncephalastrum racemosum SC47	15.1	-46.5	2.2	-34.7
Dendryphiella salina SC77	--	--	2.8	-17.0
Processed control[e]	14.3	-44.4	2.6	-22.9
Untreated material	16.7	--	2.8	--

[a] Medium used: 5% wheat bran or bagasse (ground through a 40-mesh Wiley mill screen), 0.24% NH_4NO_3, 0.006% KH_2PO_4, 0.01% yeast extract, in 25 ml. 15 o/oo seawater in 125 ml DeLong flasks.

[b] With culture collection accession numbers.

[c] Percent crude protein (= percent nitrogen x 6.25) of fermented product (wheat bran and mycelium).

[d] Crude protein of final product/crude protein of original material, x 100.

[e] Flasks of medium taken through sterilization, fermentation and harvesting process, but not inoculated.

[f] Not determined.

Table 3. Results of the second set of culture screens intended to detect strains of fungi optimally able to convert wheat bran into mycelial biomass.[a]

FUNGAL STRAIN[b]	WHEAT BRAN	
	CP%[c]	ΔCP%[d]
Myrothecium sp. SC87	34.3	+50.6
Septonema secedens SC104	29.8	+40.6
Curvularia sp. SC86	33.4	+37.9
Zythia sp. SC20	32.5	+35.9
Chaetomium sp. SC97	31.7	+34.8
Scopulariopsis (?) sp. SC8	31.0	+20.3
Aspergillus terreus SC114	31.0	+19.6
Lulworthia sp. SC73	29.9	+19.3
Melanospora sp. SC94	27.6	+17.9
Curvularia tuberculata SC91	26.3	+10.1
Aspergillus niger SC90	27.7	+ 7.7
Cephalosporium sp. SC1	28.3	- 0.3
Cladosporium cladosporioides SC 103	22.9	- 1.1
Cladosporium sp. SC102	22.0	- 2.3
Geotrichum candidum SC117	26.4	- 2.3
Alternaria longissima SC122	24.4	- 5.7
Botryosphaeria sp. RZ 254	25.5	- 7.3
Zythia sp. SC7	21.2	-11.8
Dendryphiella salina SC77	26.4	-12.0
Blakeslea trispora SC88	20.6	-15.3
Sporormia sp. SC100	23.7	-15.9
Aspergillus amstelodami SC43	21.7	-20.9
Trichoderma viride SC51	33.1	--[e]
Pestalotia sp. SC38	32.9	--
Pestalotia sp. SC89	32.5	--

Table 3. (continued)

[a] Medium used: 5% wheat bran, 0.86% NH_4NO_3, 0.09% KH_2PO_4, 0.01% yeast extract, in 50 ml 15 o/oo seawater, in 250 ml Ehrlenmeyer flasks.

[b] With culture collection accession numbers.

[c] Percent crude protein (= percent nitrogen x 6.25) of fermented product (wheat bran + mycelium).

[d] Crude protein of final product/crude protein of original material, x 100.

[e] Not determined.

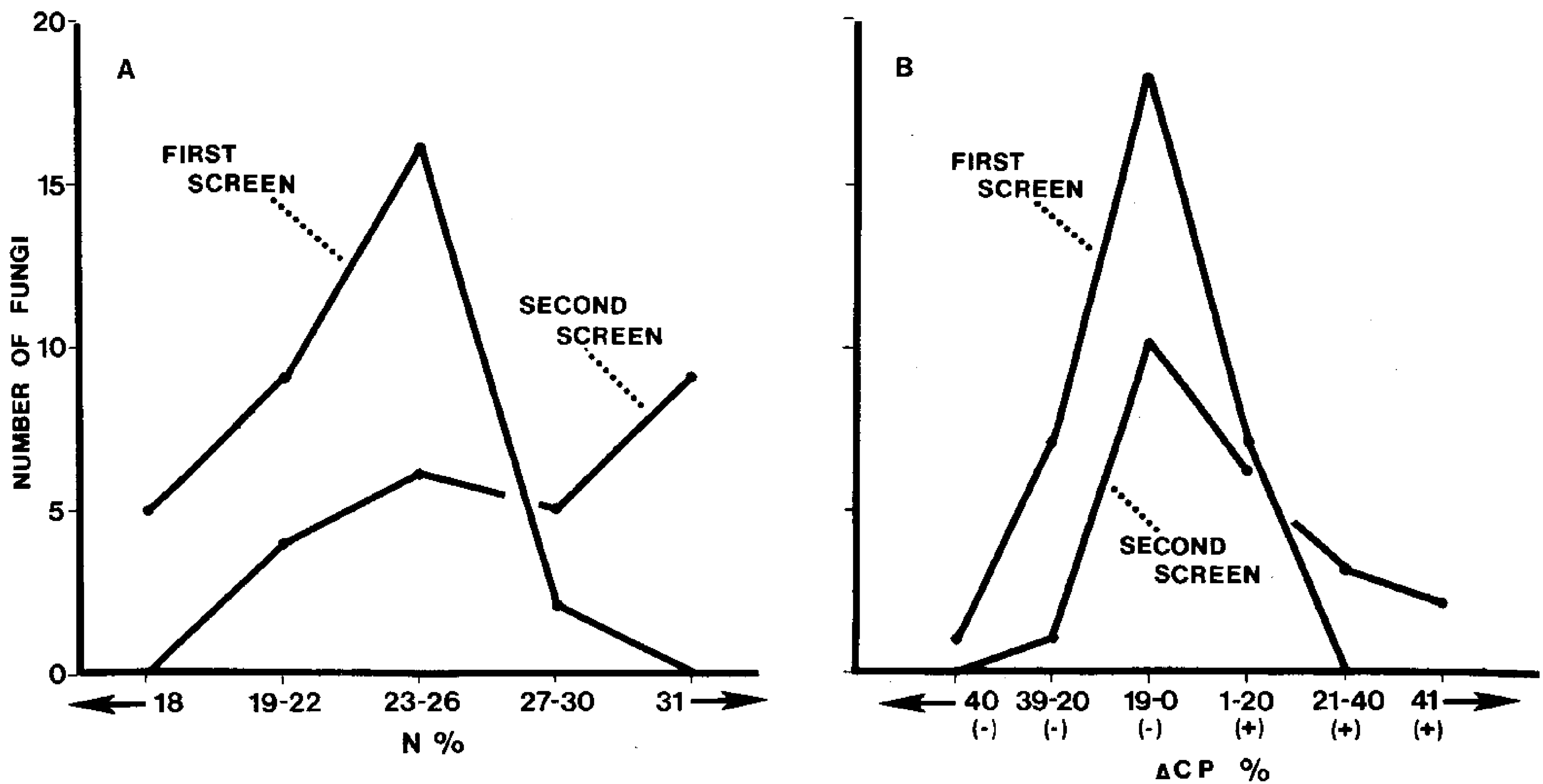

Figure 1. Distribution of screened fungi by size class of: A. nitrogen content (N%) of fermented wheat bran; B. percent crude protein change (ΔCP%) of fermented wheat bran. See Tables 2 and 3 and text for further details.

percent crude protein (Lulworthia medusa var. biscaynia, 53.7%, and Corollospora maritima, 68.9%), the highest final crude protein content of the end products was only 6.3%, considerably lower than the requirement of penaeid shrimp (Section I).

7. Production of experimental feeds

Background.

Before the crude protein analyses of the first culture screen could be completed, an opportunity arose for preliminary testing of fungal-fermented products as feed for pink shrimp. Three fungi from the culture screen were used to ferment wheat bran in 5 gallon glass carboys, using the same conditions as in the first culture screen. The products were harvested as before, dried at 50°C, ground through a 60-mesh Wiley mill screen, and fed to postlarval (average initial fresh weight - 0.11 gm) pink shrimp in the concrete tanks described above. It was unfortunate that the crude protein analyses from the first culture screen were not available during the design of this experiment, for the three fungi chosen were among the poorest performers of the screen (Syncephalastrum racemosum, Nia vibrissa, and Dendryphiella salina). Maximum final crude protein content from the carboy fermentations was 18.6%, corresponding to a net loss of 57.0% crude protein. Shrimp yield from all three fungal feeds was 1.6 times or more lower than that from unfermented bran, though all three fungal feeds gave average shrimp yields 7-8 times higher than unfed controls.

Materials and methods.

During the fall of 1973, we obtained a 14-liter laboratory fermentor (Microferm, New Brunswick Scientific Co.), with dissolved oxygen and pH controls, for the purpose of feed production for the 1974 shrimp-growing season. Only the warmer months when temperature does not fall below 20°C are suitable (e.g. Kilgen and Harris, 1975). We completed analysis of our culture screens, and identified those isolates apparently best able to convert wheat bran into a more highly proteinaceous product. Fermentor runs were planned using these fungi in order to determine how closely scaled-up fermentation processes would correspond to the flask-level fermentations, and to produce feed material for shrimp-feeding experimentation in 1974. We soon encountered the difficulty discussed by Solomons (1969), Underkofler et al. (1947), and Weismann (1970) with fermentation of particulate substrates: contamination due to the resistance to conventional steam sterilization of spore-forming bacteria, when these are protected by starch or protein components of particulate media in large volume. Rather than solve the problem by purchase of a stirring autoclave, as Weismann (1970) suggests, or by using in-place steam sterilization (impractical in our case), we found that slight modification of the autoclave and separate autoclave sterilization of all fermentation components (bran, seawater and inorganic nutrients, apparatus) was effective in preventing bacterial contamination. The autoclave modification involved venting from near the bottom rather than the top, permitting saturated steam to displace air (Ernst, 1968).

Because of the potential contamination problem, we used fast-growing (and thus contamination-resistant) fungi as fermenting agents in our production of test feeds. Other considerations here were the necessity to produce large quantities rapidly, and to reduce the projected cost of larger-scale operation (which reduction in length of fermentation time would do).

References consulted in selection of methodology include: Blakebrough (1969), Calam (1969a, b), Rowley and Bull (1973), and Solomons (1969). Inoculum was produced as follows: 250 ml Ehrlenmeyer-flask fermentations of wheat bran were performed as in the second set of wheat bran culture screens. These were harvested at early stationary phase, the entire 50 ml contents homogenized for 60 seconds as in the culture screens, and the entire homogenate added to 450 ml of the same medium in a 2000 ml Fernbach flask. This was incubated as the 250 ml stage flasks had been, harvested at early stationary phase, homogenized (in approximately 60 ml aliquots) and added aseptically to the sterilized medium in the fermentor. The fermentation was then immediately begun (agitation, sparging, pH, foam, and temperature controls adjusted and set into operation). Settings and conditions for individual runs are given in Table 4. Foam control was by automatic addition of polypropylene glycol P2000. Except when difficulties occurred with probe-sensitivity fluctuation (not shown in Table 4), only a few milliliters of the antifoam agent were added to the fermentation. All the fermentation runs were temperature-controlled at 28°C. All runs were batch fermentations; we attempted to terminate all runs in early stationary phase, as indicated by decline in oxygen uptake and rise in pH (all runs were with ammonium ion as the inorganic nitrogen source; see Prokop and Stros, 1974). Harvesting and analytical methods were similar, though on a larger scale, to those used in the culture screens. In addition, solids which passed the double-layer, 42 μm nylon-cloth filters were collected by continuous centrifugation in a Sharples T1 Super Centrifuge which was obtained at mid-year, 1974.

Results.

The four fungi which were used and the results of fermentor runs, the products of which were used in subsequent shrimp-feeding experiments, are given in Table 4. Range of content of crude protein for the four fungal products was from 25% to 32%, and increment in crude protein from -31% to +25%.

8. Attempts to optimize crude protein production

Materials and methods.

During summer, 1974, we performed four flask-scale experiments designed to determine the extent to which wheat bran fermentation results could be improved by manipulation of fermentation conditions. The experimental design was a 3-way, 3-level, 2-replicate, factorial analysis of variance (ANOVA), and each experiment tested the response of a different one of the fungi from the bran culture screens. Statistical methodology used in this experiment and the others of this communication was derived from Siegel (1956), Snedecor and Cochran (1967), Sokal and Rohlf (1969),

Table 4. Conditions and results of fermentations conducted in a 14-liter laboratory fermentor, the products of which were used as test feeds for shrimp in the F74 series (Section 9).

Run Number	Fungal Strain[a]	% Wheat Bran[b]	$\%NH_4NO_3$	Salinity, $^o/oo$	Inoculum, lts.	Inoc., Age, hrs.[c]	Base Used, ml.[d]	RPM[e]	Sparging, lts./min.[f]	Duration, hrs.[g]	Δ Dry Weight %[h]	Crude Protein %[i]	Δ C.P., %[j]
19	SC51	5	0.86	15	0.5	72	--[k]	300	1	94	37	28	+5
20	SC51	5	0.86	15	0.5	72	100	500	2	69	33	32	+25
21	SC51	5	0.65	15	0.5	72	120	500	2	73	41	31	+6
22	SC87	5	0.65	15	1.0	96	23	500	2	115	44	32	+5
24	SC51	5	0.65	15	1.0	48	139	500	2	69	-[m]	28	-[m]
25	SC87	5	0.65	15	1.0	120	0	500	2	96	40	32	+11
26	SC51	5	0.65	15	1.0	96	117	500	2	52	38	31	+13
27	SC87	5	0.65	15	1.0	120	0	500	2	98	39	31	+11
28	SC87	5	0.65	15	1.0	72	0	500	2	92	40	31	+8
29	SC86	5	0.65	15	1.0	72	0	500	2	71	37	25	-6
31	SC38	5	0.65	15	1.0	52	42	500	2	33	40	26	-9
32	SC38	5	0.65	15	1.0	96	62	700	2	37	39	27	-2
34	SC51	8	0.54[n]	5	1.0	96	63	700	4	36	54	26	-31
35	SC87	2	0.34	5	1.0	48	8	600	2	32	54	27	-27
36	SC87	2	0.34	5	1.0	48	10	600	2	33	51	26	-25

Table 4. (continued)

a Fungal strain used, given as culture collection accession numbers. SC51-Trichoderma viride; SC87-Myrothecium sp.; SC86-Curvularia sp.; SC38-Pestalotia sp. [illegible]-Pestalotia sp.

b The percentage of wheat bran used, w/v. Wheat bran was ground except for runs 35 and 36, for which it was pre-ground through 20-mesh Wiley mill screen. Runs 24-36 employed 10.5 liters of media; runs 19-24, 11 liters.

c The age at which inoculum cultures were homogenized and added to the fermentation vessel.

d The amount of 2N KOH utilized by the automatic pH control system in maintaining the pH above (not at) 4.0.

e The revolutions per minute of the fermentor impeller.

f The air flow into the fermentation vessel.

g The length of time between inoculation and harvest of the fermentation.

h Final dry weight of product (mycelium and residual bran)/initial dry weight of bran, x 100.

i Percent nitrogen of product x 6.25.

j Crude protein of product/initial crude protein of bran, x 100.

k pH maintained at 4.5 throughout fermentation.

m Data lost.

n $(NH_4)_2 SO_4$.

and Zar (1974). Two fast-growing fungi were used (Trichoderma viride SC 51 and Myrothecium sp. SC 87) and two slower-growing, marine fungi (Lulworthia sp. SC 73 and Leptosphaeria maritima RZ 312). The three treatments with their levels were: salinity, 5 o/oo, 15 o/oo, 30 o/oo; bran concentration (w/v), 2%, 5%, 8%; type of inorganic nitrogen source (w/v), high NH_4NO_3, low NH_4NO_3, and low $(NH_4)_2SO_4$. The levels of inorganic nitrogen salt used provided an equivalent carbon/ammonium ion ratio in the low NH_4NO_3 and $(NH_4)_2SO_4$ flasks ($C/NH_4 - N = 28$) and one-half that ratio for the high NH_4NO_3 flasks. The resultant percentages of the nitrogen sources were, for high NH_4NO_3: 0.34%, 0.86%, and 1.38%; for low NH_4NO_3: 0.17%, 0.43%, and 0.69%; for $(NH_4)_2SO_4$: 0.14%, 0.34%, and 0.54%. The C/NH_4-N ratio was used for standardization, rather than C/N, because of the probability that the fungi would take up the ammonium ion in preference to the nitrate ion (Nicholas, 1965) and the fact that the ammonium was probably present in large excess of requirement for maximal theoretical nitrogen content of mycelium and maximal theoretical conversion of bran (Section 6). Methods of preparation, incubation, and analysis were similar to those of the first bran culture screen, with two important exceptions: (1) inoculum level was lower than in the culture screen (Table 5; 2 ml of each homogenate were added to each 125 ml experimental flask) and the inoculum for SC 73 was less than 2 mg/flask; (2) all fermentations were terminated at 7 days, rather than at subjectively determined early stationary phase.

Due to the large numbers of flasks having to be harvested at the same time, all flasks of a given experiment were removed from the incubator at the same time, and stored at 2°C. They were then harvested on a random basis over a period of 5 days. A regression was performed of crude protein content on length of storage (days) [illegible], and slope (-0.003) was not significantly different from zero ($r = -0.045$, N.S.); the storage period did not bring about loss of protein from the fermented product.

Table 5. Inoculum data for the 3 x 3 x 3 ANOVA experiments designed to determine how the wheat bran fermentations might be optimized with regard to protein production

FUNGAL STRAINS [a]	WT	)L. [b]	#V.E.[c]
Lulworthia sp. SC 73	0.	;/ml	48
Leptosphaeria maritima RZ 312	1.	;/ml	119
Myrothecium sp. SC 87	1.	;/ml	210
Trichoderma viride SC 51	2.	;/ml	?[d]

[a] With culture collection accession number

[b] Dry weight per unit volume; 2 ml were us ıs inoculum for each experimental flask.

[c] Number of viable elements in one drop fr :he same one ml pipette, determined on cornmeal agar spreadplates

[d] Too many to count; inestimable.

Results.

ANOVA results of the four experiments are given in Tables 6-9 for nitrogen content (N%; arcsin transformation) and crude protein increment (ΔCP) of the fermentation products, and treatment means are graphed in Figs. 2-7. In each case, the treatment means are graphed only if the ANOVA indicated that the treatments had significant (p, Type I error $\leq$ 0.05) effects upon them. Values for controls (no fungal inoculation; no inorganic nitrogen addition; pure mycelium from sucrose) are given in Table 10.

Two general patterns emerge from Figs. 2-7: (1) decrease in salinity from 30 o/oo to 5 o/oo brings about increase in N% and Δ CP, and (2) high NH_4NO_3 > low NH_4NO_3 > low $(NH_4)_2SO_4$ with respect to their influence on increasing N% and Δ CP. These patterns, however, are clear in only a few instances (e.g. Fig. 4A, Fig. 3, 8% bran). Bran concentration was involved in significant interactions with other treatments in every case but one (Fig. 7); no generalizations are possible. In most cases, the interacting effects of two or all three treatments prevent generalized conclusions (e.g. Figs. 2,3, and 6).

Leptosphaeria maritima RZ 312 converted wheat bran most efficiently in 8% bran, at 5 o/oo salinity and with the high concentration of NH_4NO_3 (Figs. 2 and 3). The Δ CP% (40) was near the highest of the bran culture screen results (Table 3), and the Δ CP, mg/ml, (5.4) was higher than that of the highest of the bran culture screen results (4.3). This was in contrast to the performance of this fungus in the first bran culture screen (N% = 4.1, Δ CP % = 14.5; Table 2) at the lower concentration of NH_4NO_3 (0.24%) and KH_2PO_4 (0.006%) of that experiment.

L. maritima was the only one of the four fungi tested to show improvement over its culture screen performance. Lulworthia sp. SC 73 (Fig. 4) matched its culture screen performances (Tables 2 and 3), but it achieved this only in media of 5 o/oo salinity, as opposed to the 15 o/oo media of the culture screen. Concentration of NH_4NO_3 had no effect on N% or Δ CP, but $(NH_4)_2SO_4$ gave lower N% than NH_4NO_3

Table 6. Mean values with 95% confidence intervals of nitrogen percentage and change in crude protein content of fermented wheat bran from an experiment designed to determine how the bran fermentations might be optimized with regard to protein production. Results for Leptosphaeria maritima RZ 312, for which all experimental treatments (bran concentration, salinity, and nitrogen source) and all interactions between and among them produced significant (P, Type I error < 0.05) effects on both nitrogen percentage and crude protein change. See Figures 2 and 3.

N%[a,f]

	2% B[c]			5% B			8% B		
	S o/oo[b]			S o/oo			S o/oo		
	5	15	30	5	15	30	5	15	30
HINN[d]	3.87	3.73	4.01	4.62	4.35	4.22	4.92	4.21	4.26
LONN	4.05	3.66	4.04	4.12	4.04	4.06	4.28	4.26	4.06
LONS	3.73	3.60	4.20	4.24	4.17	4.09	4.12	3.71	3.96

ΔCP[e,f]

	2% B[c]			5% B			8% B		
	S o/oo			S o/oo			S o/oo		
	5	15	30	5	15	30	5	15	30
HINN	3.5(4%)	-0.1(0%)	3.0(4%)	65.0(31%)	43.0(20%)	32.0(15%)	136.0(40%)	41.5(12%)	6.5(2%)
LONN	4.5(5%)	-4.5(5%)	0.1(0%)	31.0(15%)	53.0(25%)	10.0(5%)	60.0(18%)	34.5(10%)	17.0(5%)
LONS	-0.1(0%)	-6.0(7%)	16.0(19%)	43.5(20%)	30.5(14%)	17.0(8%)	50.5(15%)	6.5(2%)	11.5(3%)

Table 6 (continued).

[a] Nitrogen percentage

[b] Salinity

[c] Bran concentration

[d] HINN = high NH_4NO_3 concentration; LONN = low NH_4NO_3; LONS = low $(NH_4)_2SO_4$.

[e] Change in crude protein in mgs., with percent change in parentheses.

[f] Confidence interval not computed; these means are for two replicates only.

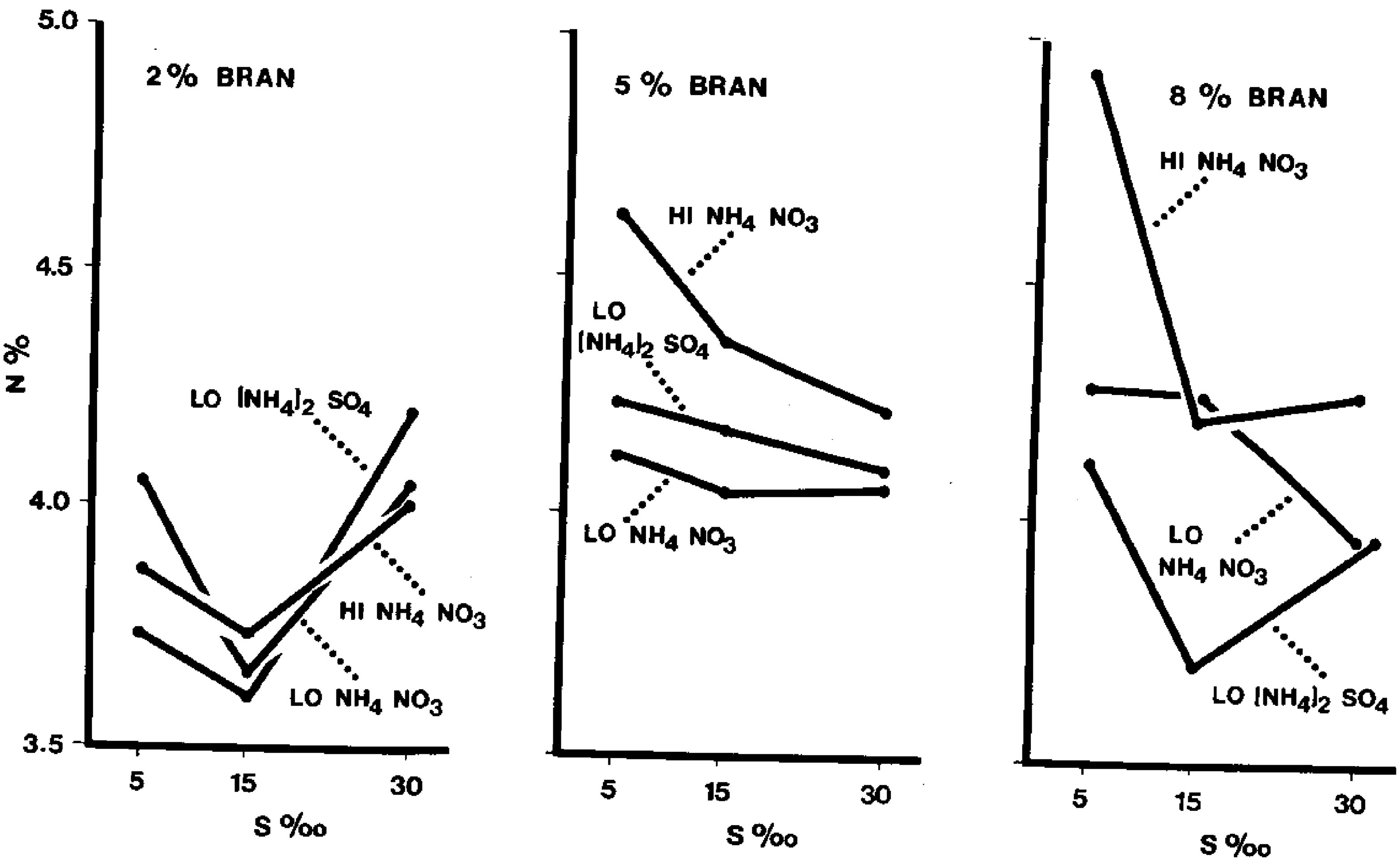

Figure 2. Leptosphaeria maritima RZ312. Means which were significantly affected by treatments (P, Type I error < 0.05). The interacting effects of bran concentration, nitrogen source, and salinity (S o/oo) on nitrogen content (N%) of fermented wheat bran. See Table 6 for further details.

Figure 3. Leptosphaeria maritima RZ312. Means which w[illegible]re significantly affected by treatments (P, Type I error < 0.05). The interacting effects of bran concentration, salinity (S o/oo), [illegible] and nitrogen source on crude protein increment (Δ CP, mgs). See [illegible] Table 6 for Δ CP%.

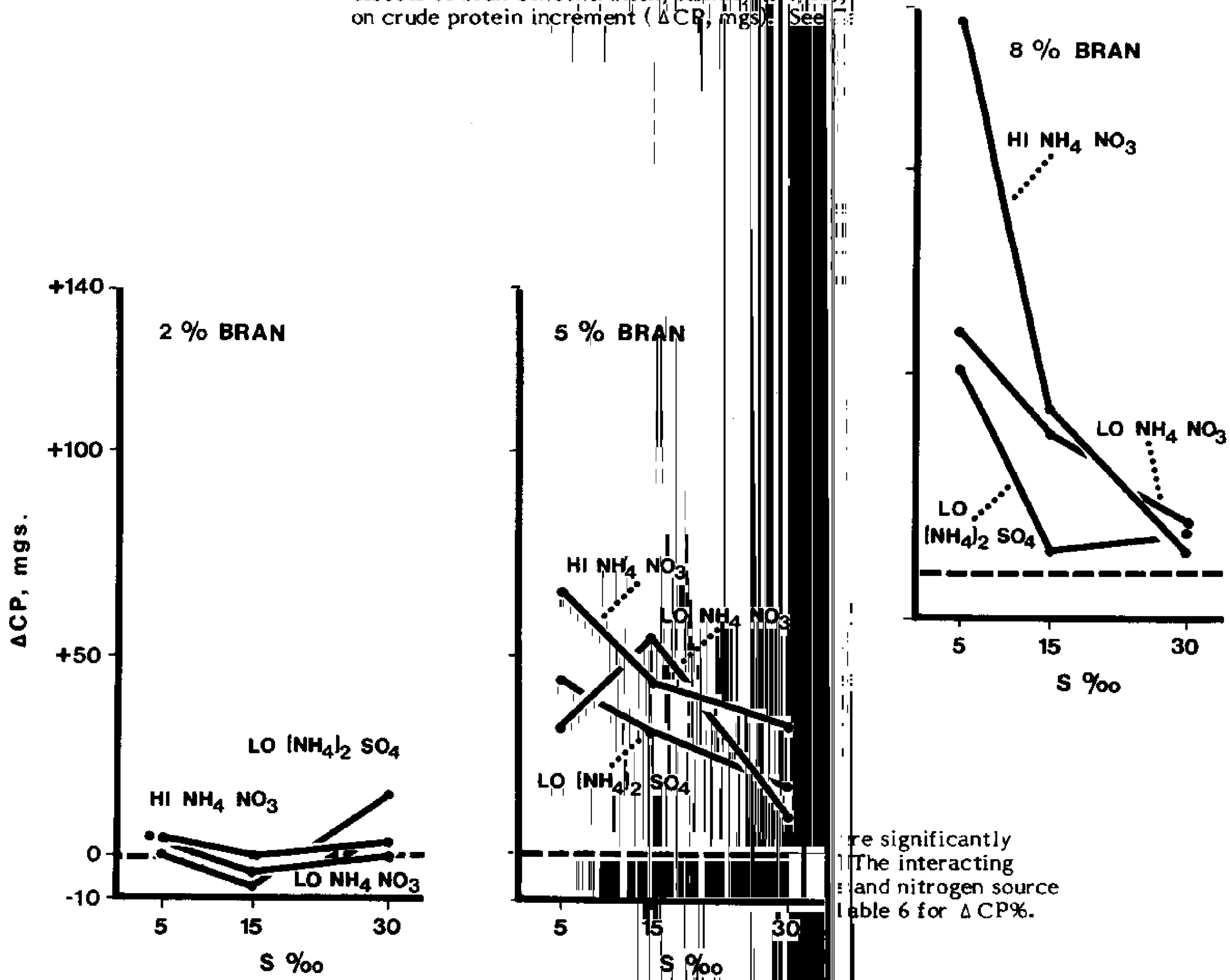

Table 7. Mean values with 95% confidence intervals of nitrogen percentage and change in crude protein content of fermented wheat bran from an experiment designed to determine how the bran fermentations might be optimized with regard to protein production. Results for Lulworthia sp. SC 73, for which bran concentration, salinity, nitrogen source, and the interaction between nitrogen source and bran concentration produced significant (P, Type I error < 0.05) effects on nitrogen percentage, and bran concentration, salinity, and the interaction of bran concentration and salinity produced significant effects on crude protein change. See Figure 4.

N %[a]

					HINN[d]	LONN	LONS
S o/oo[b]	5	4.60 ± 0.13	B%[c]	2	4.68 ± 0.31	4.67 ± 0.41	4.20 ± 0.40
	15	4.27 ± 0.18		5	4.19 ± 0.64	4.22 ± 0.33	4.17 ± 0.38
	30	3.90 ± 0.15		8	4.08 ± 0.33	4.04 ± 0.30	4.06 ± 0.40

ΔCP[e]

S o/oo

		5	15	30
B %	2	11.0 ± 2.6 (13%)	4.3 ± 9.9 (5%)	3.0 ± 4.8 (4%)
	5	33.0 ± 13.9 (15%)	16.8 ± 13.5 (8%)	-8.3 ± 6.7 (4%)
	8	30.2 ± 16.0 (9%)	-9.7 ± 13.4 (3%)	-34.5 ± 13.9 (10%)

[a] Nitrogen percentage

[b] Salinity

[c] Bran concentration

[d] HINN = high NH_4NO_3 concentration; LONN = low NH_4NO_3; LONS = low $(NH_4)_2SO_4$.

[e] Change in crude protein in mgs., with percentage change in parentheses.

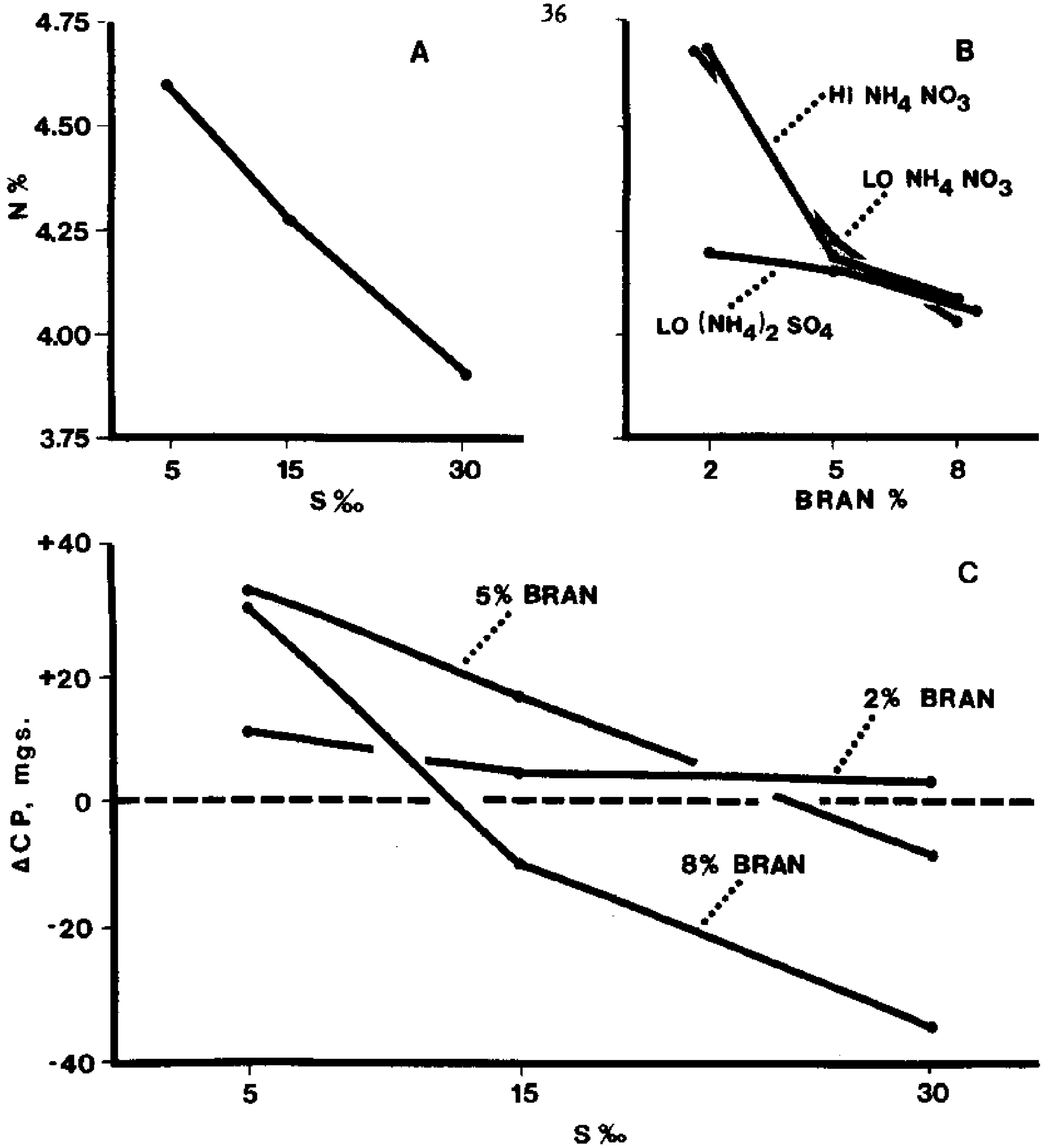

Figure 4. Lulworthia sp. SC73. Means which were significantly affected by treatments (P, Type I error < 0.05). A. The effect of salinity (S o/oo) on nitrogen content (N%) of fermented wheat bran. B. The interacting effects of bran concentration (Bran %) and nitrogen source on nitrogen content. C. The interacting effects of salinity and bran concentration on crude protein increment (Δ CP, mgs). See Table 7 for confidence intervals and % Δ CP.

Table 8. Mean values with 95% confidence intervals of nitrogen percentage and change in crude protein content of fermented wheat bran from an experiment designed to determine how the bran fermentations might be optimized with regard to protein production. Results for Myrothecium sp. SC 87, for which concentration of bran, salinity, and the interaction of bran concentration and nitrogen source produced significant (P, Type I error < 0.05) effects on nitrogen percentage, and bran concentration and the interaction between bran concentration and salinity produced significant effects on crude protein change. See Figure 5.

N %[a]

					HINN[d]	LONN	LONS
	5	4.41 ± 0.13		2	4.71 ± 0.24	4.59 ± 0.16	4.33 ± 0.11
S o/oo[b]	15	4.23 ± 0.13	B%[c]	5	4.37 ± 0.28	4.25 ± 0.21	4.37 ± 0.18
	30	4.40 ± 0.13		8	4.11 ± 0.25	4.11 ± 0.30	4.29 ± 0.10

ΔCP[e]

		S o/oo		
		5	15	30
	2	16.5 ± 8.4 (19%)	17.3 ± 8.0 (21%)	12.7 ± 8.1 (15%)
B %	5	-7.5 ± 23.0 (4%)	1.8 ± 8.8 (1%)	9.8 ± 7.6 (5%)
	8	0.3 ± 20.5 (0%)	-27.2 ± 22.0 (8%)	-15.3 ± 11.1 (5%)

[a] Nitrogen percentage

[b] Salinity

[c] Bran concentration

[d] HINN = high NH_4NO_3 concentration; LONN = low NH_4NO_3; LONS = low $(NH_4)_2 SO_4$.

[e] Change in crude protein in mgs., with percentage change in parentheses.

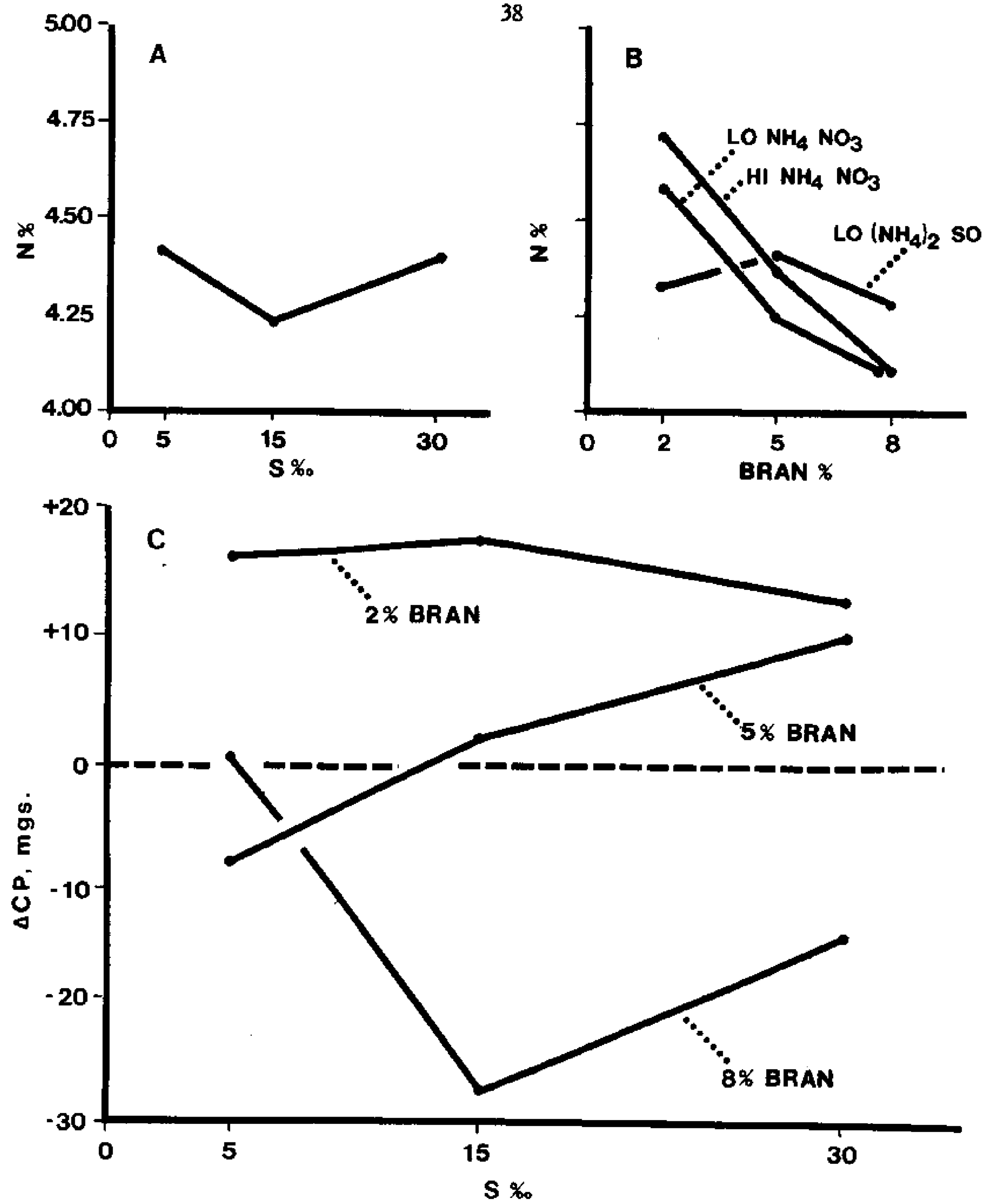

Figure 5. *Myrothecium* sp. 5C87, Means which were significantly affected by treatments (P, Type I error < 0.05). A. The effect of salinity (S o/oo) on nitrogen content (N%) of fermented wheat bran. B. The interacting effects of bran concentration (Bran %) and nitrogen source on nitrogen content. C. The interacting effects of salinity and bran concentration on crude protein increment (ΔCP, mgs). See Table 8 for confidence intervals and % ΔCP.

Table 9. Mean values with 95% confidence intervals of nitrogen percentage and change in crude protein content of fermented wheat bran from an experiment designed to determine how the bran fermentations might be optimized with regard to protein production. Results for <u>Trichoderma viride</u> SC 51, for which bran concentration, salinity, the interaction between bran concentration, salinity, and nitrogen source produced significant (P, Type I error < 0.05) effects on nitrogen percentage, and bran concentration and nitrogen source produced significant effects on crude protein change. See Figures 6 and 7.

N %[a,f]

	2% B[c]			5% B			8% B		
	S o/oo[b]			S o/oo			S o/oo		
	5	15	30	5	15	30	5	15	30
HINN[d]	3.94	3.59	3.72	3.95	3.82	4.00	4.07	4.04	3.96
LONN	3.79	3.94	3.95	3.90	3.57	3.72	3.87	3.73	3.99
LONS	3.56	3.54	4.04	4.17	3.84	3.89	4.18	4.02	3.84

ΔCP[e]

B %	2	-17.9 ± 3.2 (21%)	HINN	-32.8 ± 7.7 (15%)
	5	-37.0 ± 5.7 (17%)	LONN	-40.1 ± 11.2 (19%)
	8	-49.3 ± 9.3 (14%)	LONS	-31.3 ± 8.0 (15%)

[a] Nitrogen percentage

[b] Salinity

[c] Bran concentration

[d] HINN = high NH_4NO_3 concentration; LONN = low NH_4NO_3; LONS = low $(NH_4)_2SO_4$.

[e] Change in crude protein in mgs., with percentage change in parentheses.

[f] Confidence interval not computed; these means are for two replicates only.

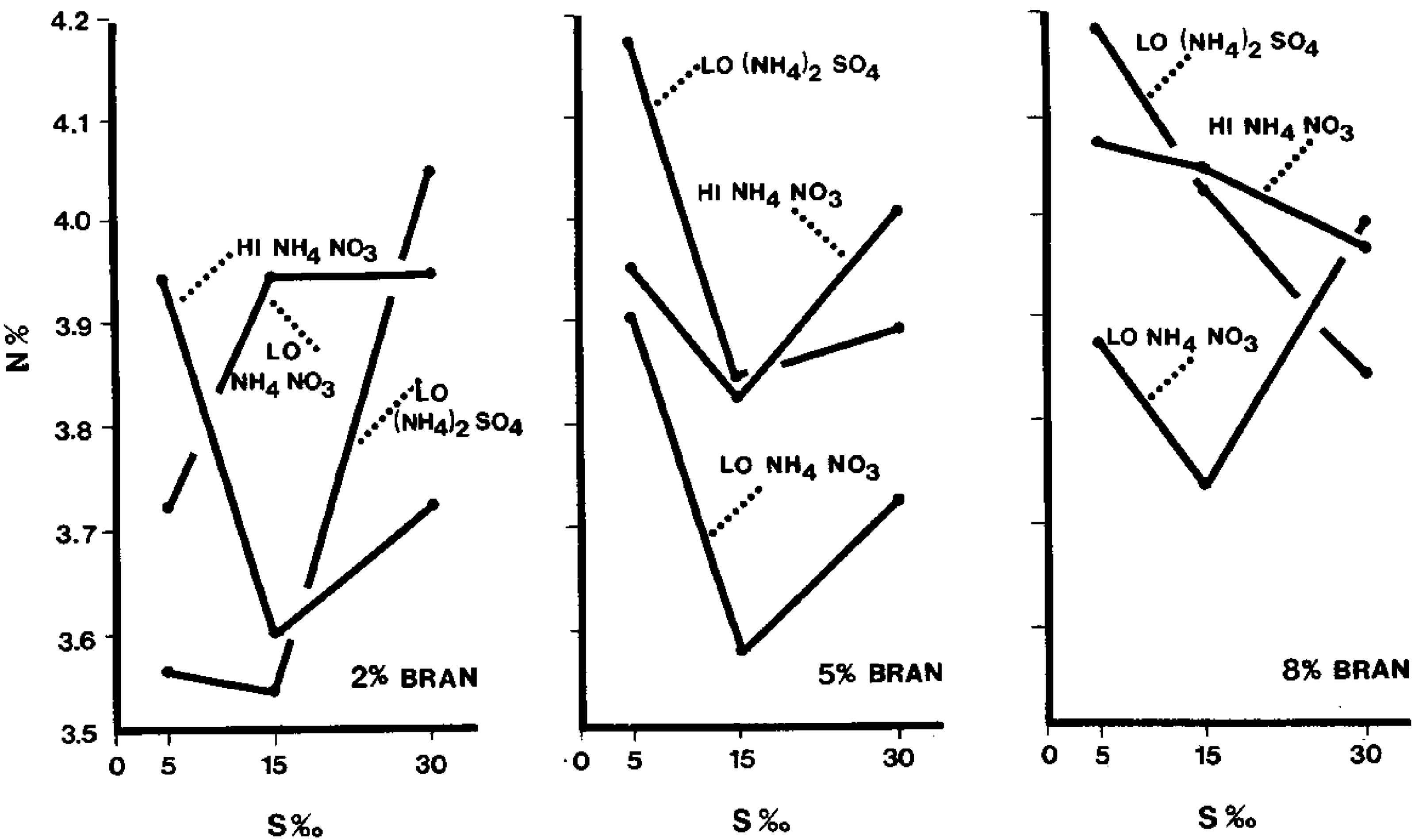

Figure 6. Trichoderma viride SC51. Means which were significantly affected by treatments (P, Type I error < 0.05). The interacting effects of bran concentration, nitrogen source, and salinity (S o/oo) on nitrogen content (N%) of fermented product. See Table 9 for further details.

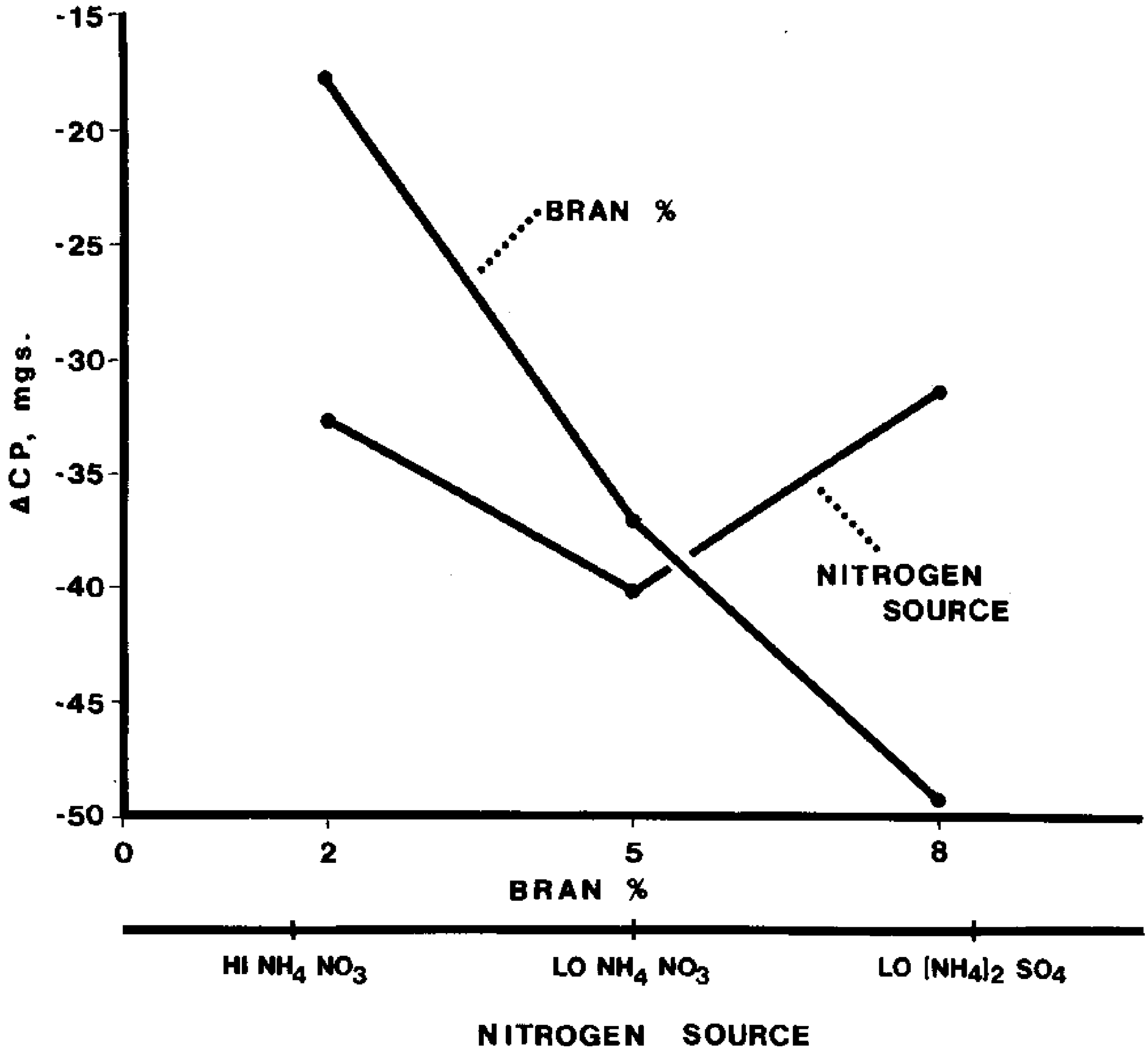

Figure 7. Trichoderma viride SC51. Means which were significantly affected by treatments (P, Type I error < 0.05). The effects of bran concentration (Bran%) and nitrogen source on loss of crude protein (ΔCP, mgs). See Table 9 for confidence intervals and %Δ CP.

at the 2% level of bran concentration, where N% produced with NH_4NO_3 was highest. At the optimal salinity level of 5 o/oo, 2% bran gave lower Δ CP than 5 and 8% bran.

Myrothecium sp. SC 87 which had exhibited the highest N% and Δ CP of the culture screens (Tables 2 and 3), gave much lower N% and Δ CP in its 3 x 3 x 3 ANOVA experiment. Its highest significantly affected mean N% was 4.7 at 2% bran and the higher NH_4NO_3 concentration. As with Lulworthia sp., $(NH_4)_2SO_4$ gave a lower N% than NH_4NO_3 at this bran concentration. The 5 o/oo and 30 o/oo salinities gave higher N% than 15 o/oo. Highest Δ CP, 17.3 mg (21%), was given by 2% bran, and there appeared to be little effect of salinity at this bran concentration though it had marked effects at the 5 and 8% bran levels. Nitrogen source had no effect on Δ CP.

As in the case of Myrothecium sp., Trichoderma viride SC 51 (Fig. 6) exhibited much lower N% (maximum mean of 4.2%) in the 3 x 3 x 3 ANOVA experiment than it had in the second culture screen (5.3%; Table 3), though its response in the first culture screen (4.0%; Table 2) was lower than its ANOVA experiment maximum. In most cases, the higher NH_4NO_3 concentration gave higher final N%, though this situation was reversed at 15 - 30 o/oo salinity with 2% bran and not apparent at 5 o/oo - 5% and 30 o/oo - 8%. At all three bran concentrations, highest N% was given by $(NH_4)_2SO_4$; this was at 30 o/oo salinity with 2% bran, and at 5 o/oo with 5 and 8% bran. The Δ CP values were all negative (Fig. 7); increasing bran concentration led to greater loss of CP and the low concentration of NH_4NO_3 brought about greater loss than either the high NH_4NO_3 or $(NH_4)_2SO_4$.

Visual observations made during harvest of the flasks included the following. The $(NH_4)_2SO_4$ flasks of neither SC 73 nor RZ 312 exhibited the marked clearing of the liquid phase evident in the NH_4NO_3 flasks. There was a marked increase in conidium formation by SC 51 as the percentage of bran decreased (the washes from the 2% bran fermentation were bright green). As the percentage of bran increased, the size of the mycelial pellets formed by SC 87 decreased markedly.

Since neither Myrothecium sp. nor Trichoderma viride performed in a fashion corresponding to its bran culture screen results, it is possible that these discrepancies were due to the standardized harvest time (7 days) of the 3 x 3 x 3 ANOVA experiment. Both of these fungi are fast-growing (Table 5). As can be seen from Table 4, maximum Δ CP% for Trichoderma viride from Microferm runs was +25%, at a bran concentration, nitrogen source and level, and salinity which gave negative Δ CP (> 10%) in the ANOVA experiment. When the set of conditions indicated as optimal for N% and Δ CP by the ANOVA experiment were tried in a Microferm run with T. viride, results quite comparable to the ANOVA results were obtained (N% = 4.1 and Δ CP = -31%) (Tables 4 and 9 and Figs. 6 and 7). This suggests that the ANOVA experiment showed best results for that combination of treatment levels which caused the fungus to grow less quickly into the later stationary phase (when conidium production--conidia are not efficiently harvested by 42 μ m filtration--and other loss of cell nitrogen takes place). Comparison of runs 35 and 36 (Table 4) with Table 8 shows another distinct lack of correspondence, but in this case, the cause is not clearly a time-of-harvest problem as with T. viride; Microferm runs 22, 25, and 27 (Table 4) for Myrothecium sp. did not correspond with the culture screen results either (Table 3), so that scale-up difficulties of some sort appear to have been involved with this fungus. Likely candidates as sources of these difficulties are differences in autoclaving of materials and in availability of dissolved oxygen during fermentation.

The control figures for N% and Δ CP (Table 10) appear to show that the products from flasks with NH_4NO_3 without fungi lost more CP and were lower in N% than those with $(NH_4)_2SO_4$, no inorganic nitrogen, and no inorganic nitrogen + Lulworthia sp. However, the 5 means for neither N% nor Δ CP are significantly different from one another (1-way ANOVA). It is interesting to note that when no

Table 10. Control values for the 3 x 3 x 3 ANOVA experiments designed to determine how the wheat bran fermentations might be optimized with regard to protein production.

8 %, 15 o/oo, No Fungi[a]

	HINN[b]	LONN	LONS	NON
% N[c]	2.91	3.04	3.16	3.15
ΔCP[d]	-106.0 (-31%)	-93.0 (-27%)	-77.5(-23%)	-77.5 (-23%)

8 %, 15 o/oo, Non, Lulworthia sp. SC 73

% N	3.10
ΔCP	-66.5 (-20%)

2 % Sucrose, 15 o/oo, 0.45 % NH_4NO_3

	RZ 312[e]	SC 73	SC 51	SC 87
N %	3.12	3.10	4.31	7.38

[a] Flasks treated like all others in the experiment, except that no fungal inoculum was added; medium of 8% wheat bran and 15 o/oo salinity; other components as in all other flasks.

[b] HINN = 1.38% NH_4NO_3; LONN = 0.69% NH_4NO_3; LONS = 0.54% $(NH_4)_2SO_4$ Non = no added nitrogen.

[c] %N = nitrogen percentage in washed and dried product.

[d] Change in crude protein from original (mg.) with percent change in parentheses.

[e] Culture collection accession numbers for fungi used; see text for binomials.

inorganic nitrogen was added, no increase in N% or prevention of loss of CP took place with Lulworthia sp. (though the fungus obviously grew). This may indicate that the protein which is solubilized during autoclaving of the wheat bran is largely unavailable, at least to Lulworthia sp. It is evident from comparison of N% for mycelium controls (in which C/NH_4 - N = that of the bran media with the high concentration of NH_4NO_3) with the N% of corresponding bran flasks, that the mycelium of some of the fermented bran products must have had higher N% than the mycelial controls. This is especially true in the case of Leptosphaeria maritima RZ 312 (unfermented wheat bran, N% = 2.67; mycelial control, RZ 312 N% = 3.12; in 8% bran, high NH_4NO_3, 5 o/oo salinity, RZ 312 N% = 4.92).

9. Feeding tests, 1974

Materials and methods.

In June, 1974, we began a series of four feeding experiments with penaeid shrimp (pink - Penaeus duorarum, and white - Penaeus setiferus). The same concrete tanks as used by Caillouet et al. (1975b) were used (Section 5). The layout of the Turkey Point shrimp mariculture facility where these experiments were conducted is given by Tabb et al. (1969) and updated by Caillouet et al. (1973). In all four of our 1974 artificial detrital feeding experiments (series F74), water was obtained from adjoining South Biscayne Bay and filtered through 5 μm filter-cloth bags into the concrete tanks. The tanks were not prefertilized and the water was not aged. Flowing water systems were not used. Overnight aeration was conducted only when dissolved oxygen concentration approached 3 ppm (which it did only twice, during F74A). All shrimp used in the experiments were hatched and raised through their larval stages at the Turkey Point nursery according to the methodology described by Tabb et al. (1972). In each experiment fungal-fermented wheat bran products were tested as feed, with controls of unfermented bran (all experiments), commercial flaked feed (F74A, F74D), fertilization with inorganic fertilizer (F74C, F74D), and no feed at all (F74A, F74B). The feeds were always thoroughly wetted with tapwater before they were added to feeding tanks, so that they would immediately sink and become available to the shrimp. All fermented feeds were prepared in the Microferm fermentor and processed as described above (Section 7). In most cases, fermentation products were dried at 55°C and ground either by mortar and pestle or in a Wiley mill. When this was not the case, products were harvested and washed as usual, but then refrigerated rather than dried.

Results.

The results of the F74 Experiments are given in Tables 11 and 12 and Figs. 8-11. Average final fresh weights (AFFW) were analyzed by 1-way ANOVA followed by Student-Newman-Keuls (SNK) least significant range method of multiple comparison among means, with confidence level = 95%. Logarithmic transformation was necessary for F74A and F74B, in which variance was inhomogeneous (Bartlett's test, p = 0.05). AFFW was approximately equal to average fresh weight gain in experiments F74A, B, and D due to the very small average initial weights of these experiments. Fresh weight gains were used in the statistical analysis of F74C, since the average initial fresh weight in that experiment was much larger than in the others.

One of the clearest conclusions to be derived from these results is that there were differences from tank to tank not due to intended treatments. These often caused marked, statistically significant differences in shrimp yields and AFFW. For this reason, means are not calculated for combined results of replicate tanks. Rather, replicate results are shown separately, so that the range of mean results is perceivable. Two major possible reasons for the unintended differences among tanks are differences in nutrient retention among tanks following previous experiments, and differences in the meiofauna and microbial flora (autotrophic and heterotrophic) which developed along with the shrimp, due to inoculum differences and nutrient retention differences. Yield values in Tables 11 and 12 are fresh weights, and feed

Table 11. Results of shrimp-feeding experiments in the F 74 Series: F 74A and F 74B, with pink shrimp (Penaeus duorarum). Treatments are identified in Table 13. See Figures 8 and 9.

	F 74A	Ave. final wt.	Yield g/m^2	Survival %	Feed efficiency	F 74B	Ave. final wt.	Yield g/m^2	Survival %	Feed efficiency
Duration, days	58					60				
Salinity range, o/oo	32-38					35-38				
Temperature range, ^{o}C	26-31					27-31				
Stocking density/m^2	15					15				
Ave. initial wt, g	0.010					0.005				
Treatment	1	0.29	4.1	93	--	1	0.35	3.6	70	--
	1	0.17	2.2	83	--	1	0.21	2.2	70	--
	2	1.19	16.1	90	9	2	0.94	10.8	77	14
	2	0.92	12.9	93	11	2	1.27	14.6	77	10
	3	0.72	10.8	100	13					
	3	0.48	6.2	87	23					
	4	1.35	20.2	100	7					
	4	1.27	15.2	80	10					
	5	0.54	7.2	90	20					
	5	0.40	5.6	93	26					
	6	0.76	11.3	100	13					
	6	0.60	8.7	97	17					
	7	0.46	6.9	100	21					
	7	0.47	7.0	100	21					
	8	0.29	2.5	57	30					
	8	0.29	2.9	67	50					
	9	0.31	4.6	97	32	10	0.62	7.5	80	20
	9	0.43	6.4	100	23	10	1.02	11.7	77	13

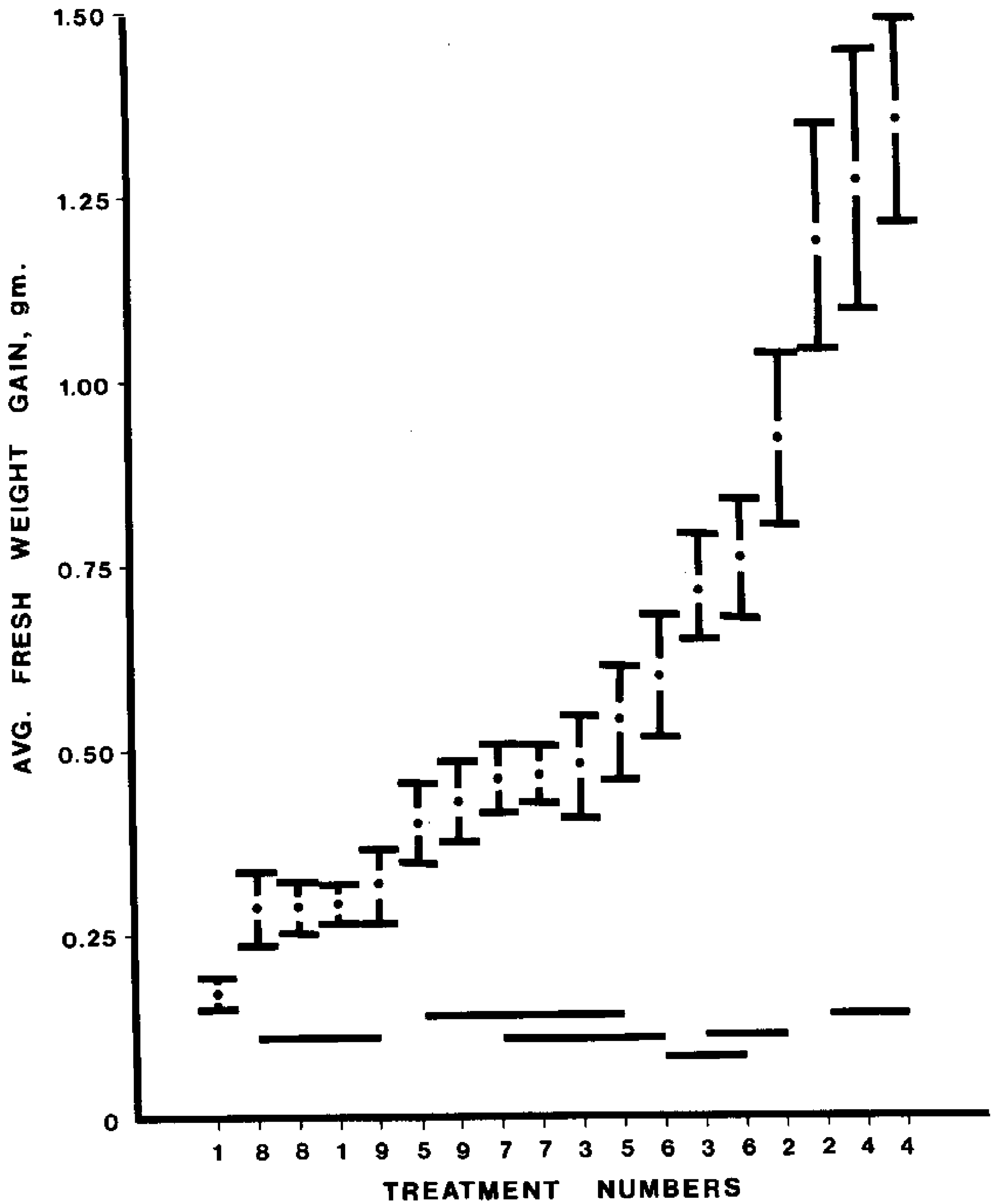

Figure 8. Average final fresh weights, with 95% confidence intervals, of pink shrimp from feeding experiment F74A. Feeding treatments: 1-no feed; 2-unfermented wheat bran; 3-autoclaved wheat bran; 4-Purina shrimp chow; 5-wheat bran fermented by Trichoderma viride SC51 (dried); 6-treatment #5, 90%, #4, 10%; 7-10% level, treatment #4; 8-treatment #5 (undried, cold-stored); 9-wheat bran fermented by SC87 (dried). Bars at base of graph join those means which could not be shown to be significantly different from one another (P, Type I error >0.05). Details in text and Table II.

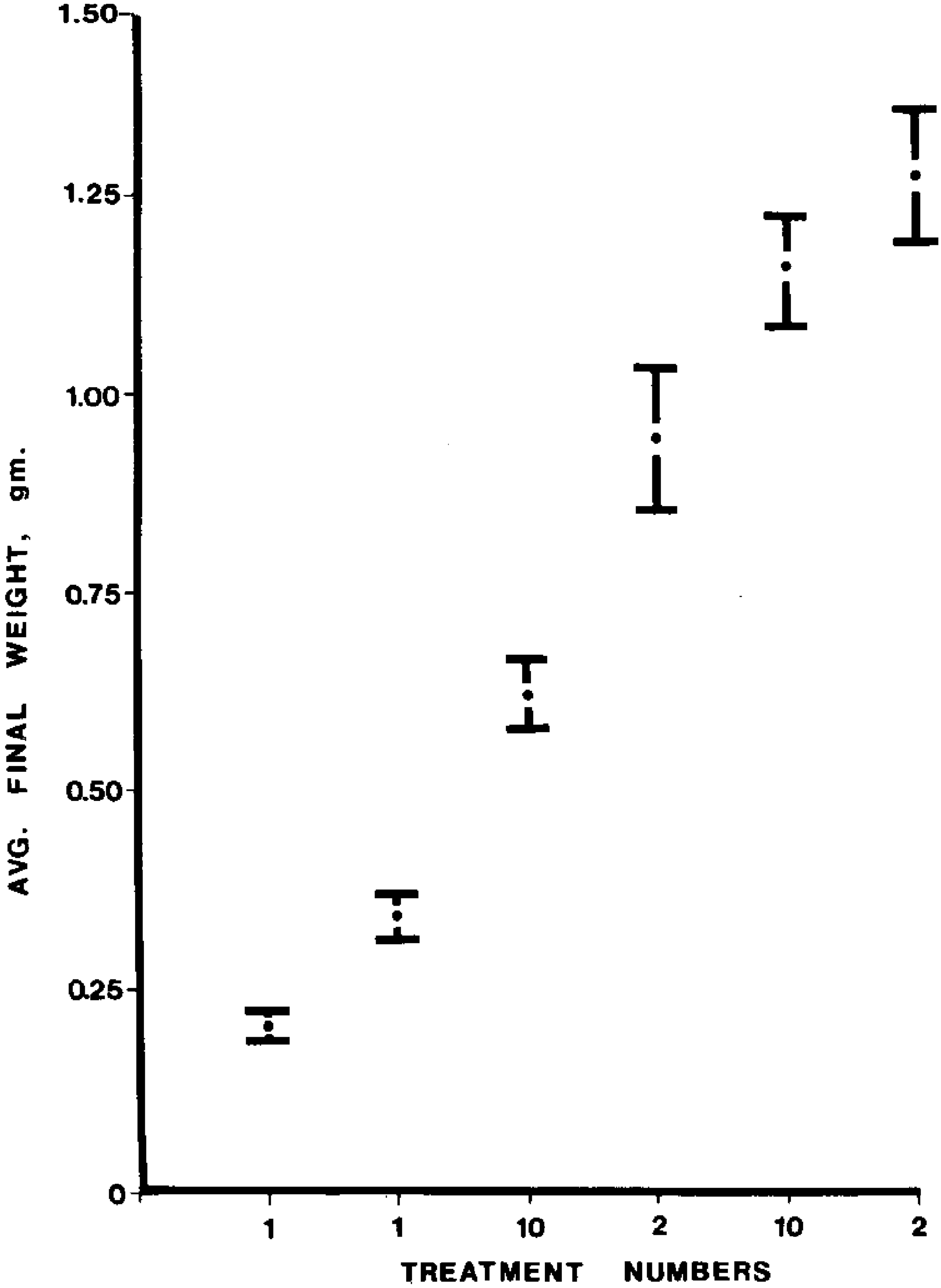

Figure [illegible]. Average final fresh weights with 95% confidence intervals of pink shrimp from feeding experiment F74B.--Feeding treatments: 1-no feed; 10-wheat bran fermented by <u>Myrothecium</u> sp. SC87 (undried, cold-stored); 2-unfermented wheat bran. All mean weights were significantly different from one another (P, Type I error<0.05). Details in text and Table II.

Table 12. Results of shrimp-feeding experiments in the F 74 Series: F 74C, with white shrimp (*Penaeus setiferus*) and F 74D, with pink shrimp (*P. duorarum*). Treatments are identified in Table 13. See Figures 10 and 11.

	F 74C	Ave. wt. gain, g.	Gain, g/m^2	Survival, %	Feed efficiency	F 74D	Ave. final wt.	Yield, g/m^2	Survival %	Feed efficiency
Duration, days	60					65				
Salinity range o/oo	35-41					33-45				
Temperature range, °C	21-26					15-24				
Stocking density/m^2	5					7.5				
Average initial wt, g	3.27					0.017				
Treatment	11	0.63	2.5	80	--	11	0.73	2.9	53	--
	11	0.54	2.7	100	--	11	0.97	1.5	20	--
	2	2.59	9.1	70	8	2	0.81	5.3	87	16
	2	1.85	9.2	100	8	2	Lost	--	--	--
	12	1.26	6.3	100	12	4	1.23	8.0	87	11
	12	1.03	5.2	100	14	4	1.11	6.6	80	13
	13	2.07	9.3	90	8	15	0.79	5.1	87	17
	13	1.39	6.3	90	12	15	0.84	4.2	67	20
	14	0.74	2.9	80	26	16	0.68	4.1	80	24
	14	0.45	2.3	100	33	16	0.57	4.0	93	24
						17	0.79	3.5	60	28
						17	0.67	4.0	80	24

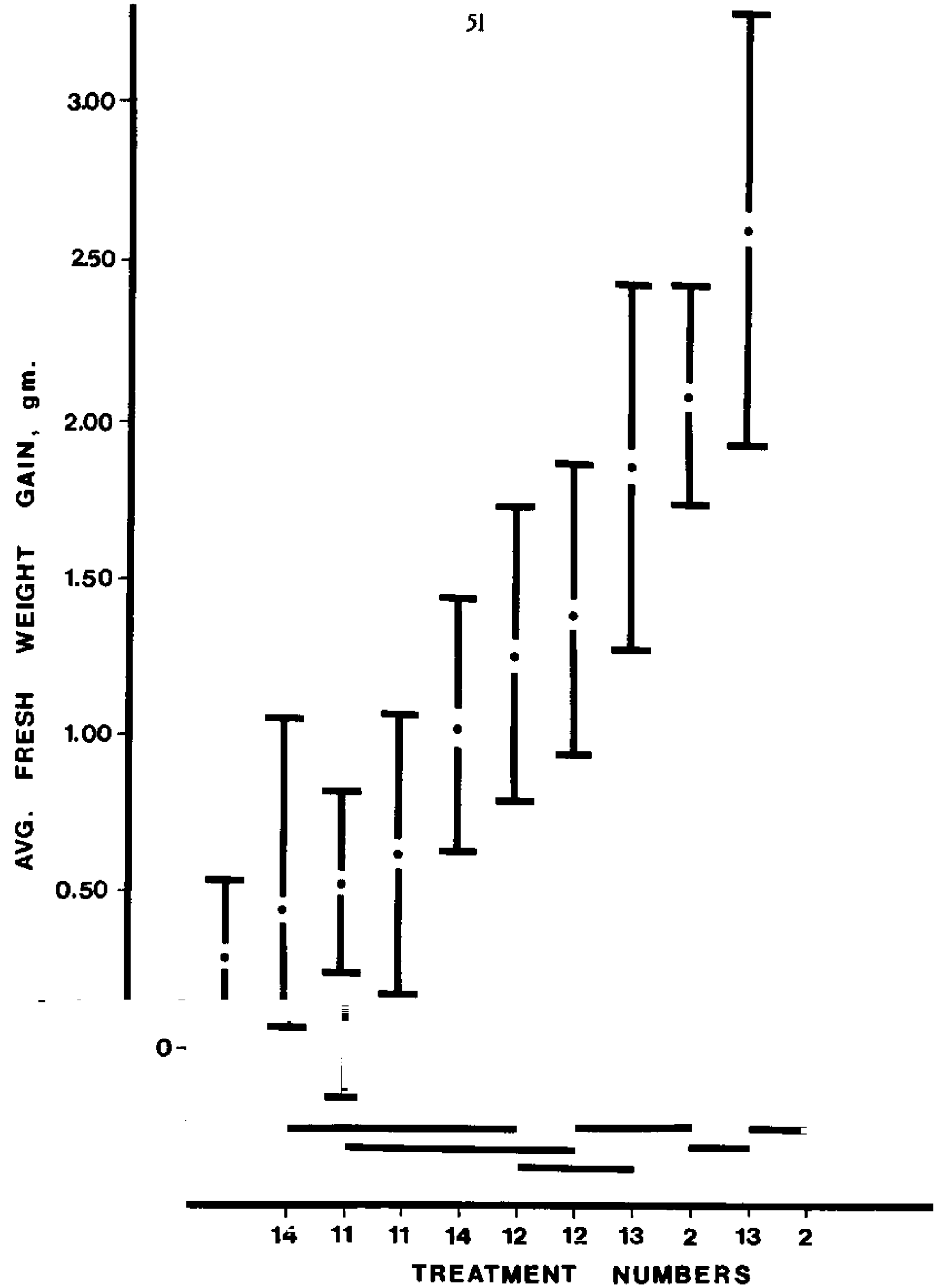

Figure 10. Average fresh weight gains, with 95% confidence intervals, of white shrimp from feeding experiment F74C. Feeding treatments: 11-regular fertilization; 2-unfermented wheat bran; 12-wheat bran fermented by Pestalotia sp. SC38 (dried); 13-treatment #3, 50%, treatment #2, 50%; 14-wheat bran fermented by Curvularia sp. SC86 (dried). Bars at base of graph join those means which could not be shown to be significantly different from one another (P, Type I error > 0.05). Details in text and Table 12.

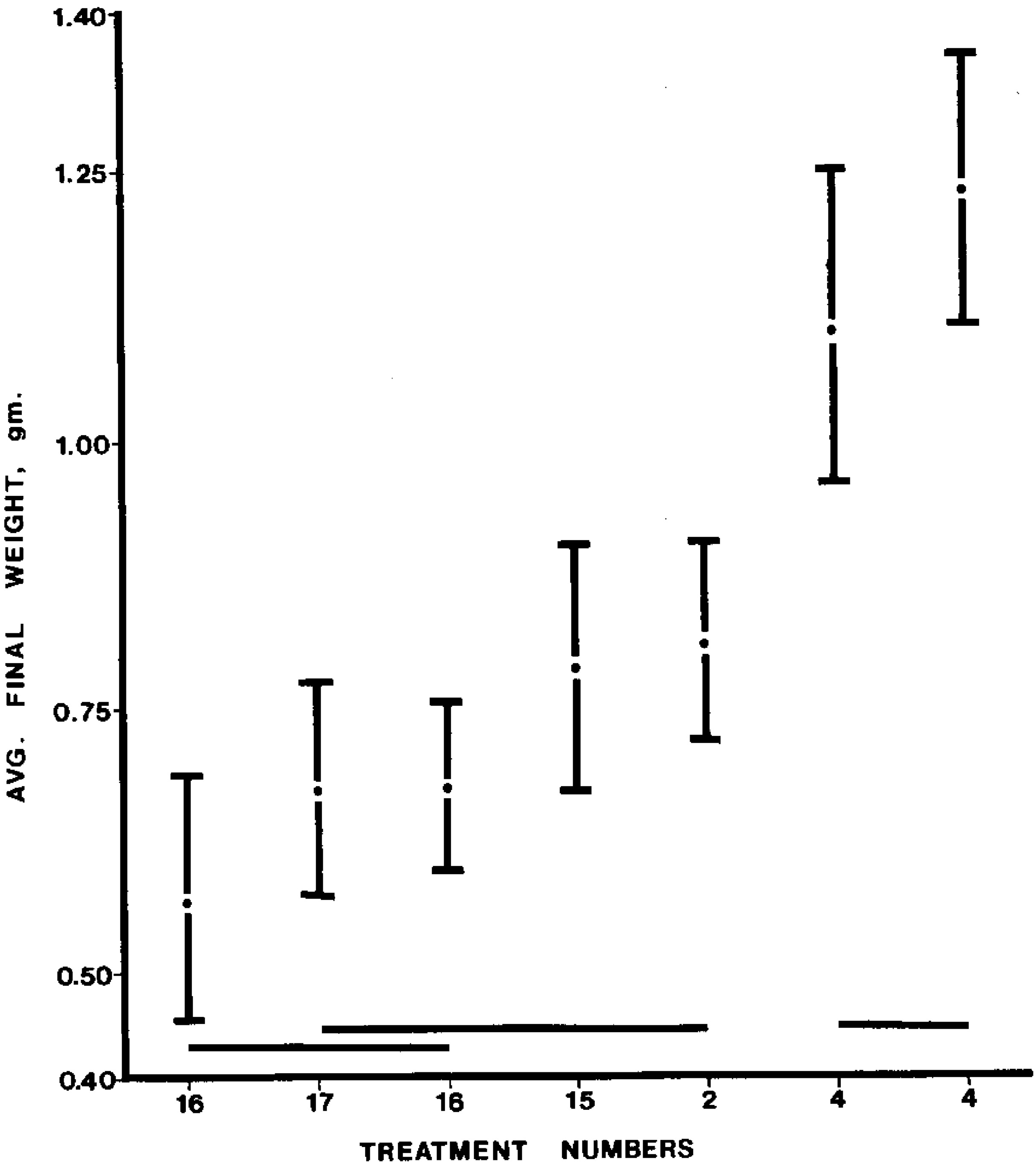

Figure 11. Average final fresh weights, with 95% confidence intervals, of pink shrimp from feeding experiment F74D. Feeding treatments: 2-unfermented wheat bran; 4-Purina shrimp chow; 15-wheat bran fermented by Myrothecium sp. SC87 (undried, cold-stored), 50%, treatment 3, 50%, 16-wheat bran fermented by Trichoderma viride [illegible] 2, 45% [illegible] 50% [illegible] 57% treatment 3, 53% (total ground through 20 [illegible] dried, 57% [illegible] 17-like treatment 5, but ground through [illegible] 60-mesh screen. Bars at [illegible] ase of graph join those means which [illegible] could not be shown to be significantly different from one another (P, Type I error > 0.05). [illegible] etails in text and Table 12.

Table 13. List of treatments used in shrimp-feeding experiments of the F74 series. Compare with Figures 8-11 and Tables 11 and 12. Except where otherwise indicated, feeding levels were equal on a dry weight basis within experiments.

1. No feeding or fertilization.

2. Unfermented wheat bran. F74A, F74B: not ground; F74C, F74D: ground (Wiley mill) through 20-mesh screen. Mean CP% = 16.7.

3. Wheat bran, autoclaved, washed, and dried as in fermented wheat bran treatments, but not inoculated or fermented; ground in mortar and pestle. Mean CP% = 18.9.

4. Purina 'shrimp chow' ('Experimental Marine Ration 20'); fed as thick flakes as received. Ingredients include fish meal, soybean meal, ground wheat, brewer's yeast, dried whey, soybean oil, dicalcium phosphate, iodized salt, vitamins, minerals. CP% = 21.8.

5. Wheat bran fermented by *Trichoderma viride* SC 51. Dried at 55°C and ground by mortar and pestle. See text for CP% and ΔCP%.

6. Treatment #5, 90%, and treatment #4, 10%.

7. Treatment #4, at 10% of its feeding level.

8. Treatment #5, but refrigerated rather than dried.

9. Wheat bran fermented by *Myrothecium* sp. SC 87. Dried at 55°C and ground by mortar and pestle.

10. Treatment #9, but refrigerated rather than dried. See text for CP% and ΔCP%.

11. Fertilization by daily addition of a commercial fertilizer (18-24-16) such that daily addition of nitrogen was equivalent to that of the most nitrogenous of the other feeds in an experiment; pre-dissolved.

12. Wheat bran fermented by *Pestalotia* sp. SC 38; dried at 55°C, ground (Wiley mill) through 20-mesh screen. See text for CP% and ΔCP%.

13. Treatment #12, 50%, and treatment #2, 50%.

14. Wheat bran fermented by *Curvularia* sp. SC 86; dried at 55°C, ground (Wiley mill) through 20-mesh screen. See text for CP% and ΔCP%.

Table 13 (continued)

15. Treatment #10, 50%, and treatment #2, 50%.

16. Treatment #5 (but ground (Wiley mill) through 20-mesh screen), 57%, treatment #2, 43%. Fed 13% more/day than other treatments in F74D. See text for CP% and ΔCP% of fermented portion.

17. Like treatment #16, but the fermented portion ground (Wiley mill) through a 60-mesh screen . Fed 13% more/day than other treatments in F74D. See text for CP% and ΔCP% of fermented portion.

efficiency = total dry weight of feed ÷ total fresh weight of animals. The treatments used in the four feeding experiments are identified in Table 13.

Experiment F74A was begun in June and harvested in August (Table 11). Wheat bran fermented by Trichoderma viride SC 51 and by Myrothecium sp. SC 87 was tested as feed for postlarval pink shrimp. The fermented materials came from Microferm runs 19-22 and 24-26. As Table 4 shows, these materials varied in N% from 28.3 to 31.8 and in Δ CP% from +5 to +25 for SC 51, and in Δ CP% from +5 to +11 for SC 87 with N% = 31.6. The SC 51 material was fed both dried (mortar and pestle ground) and refrigerated. Other feeding treatments were unfermented, unground wheat bran, autoclaved, washed, dried and ground wheat bran, commercial flaked feed ("shrimp chow," Purina Experimental Marine Ration 20), a combination treatment of 90% SC 51 fermented bran and 10% commercial flakes, a commercial flakes treatment at 10% of the feeding level of the above flake treatment, and a no-feed control. The experimental feeds were fed at 5 gms per day on a dry weight basis.

The commercial flakes performed best in AFFW in both replicates (Fig. 8), although the higher of the unfermented wheat bran replicates was not significantly different from those of the commercial pellet replicates (lower than the highest one by a factor of only 0.9). The autoclaved and washed bran replicates were both lower in AFFW and total yield than the unfermented bran replicates, although one of the replicates of each fell in a group of three means which were not significantly different from one another. Thus, autoclaving of wheat bran does not, in itself, impart higher nutritive capacity to bran.

The higher AFFW of the replicates of the dried T. viride fermented-bran feed was only slightly higher than the AFFW of the higher replicate of the Myrothecium sp. product (significant difference was not detected between them), and both AFFW's were lower than the higher of the unfermented bran AFFW's by a factor of about 0.5. Both replicates of the refrigerated T. viride feed were as low in AFFW as the higher of the no-feed replicates, lower by a factor of 3 than the unfermented bran replicates. In total yield, the refrigerated T. viride feeds were lower than the higher of the no-feed controls, due to the markedly lower survivals, indicating a possible toxicity of the refrigerated T. viride material. It must be noted here that refrigeration problems apparently occurred during storage of this feed material, for bacterial spoilage was quite noticeable by the time of harvest for F74A. The addition of 10% of commercial flakes to the dried T. viride feed produced marked improvement in AFFW and total yield; the higher of the AFFW's of the mixed-material replicates was significantly different from and 1.4 times greater than that of the higher of the unmixed replicates, although it was still smaller than that of the higher of the unfermented bran replicates by a factor of 0.6. That this improvement was not due to the commercial flake fraction alone, is shown by the fact that the AFFW of the higher of the replicates of the diet which consisted only of commercial flakes (at 10% of the daily feeding level of the other diets) was significantly lower than the AFFW of the higher of the mixed-diet replicates (by a factor of 0.6).

In July, experiment F74B was initiated. Experimental conditions were the same as those of F74A (Table 11), with one important exception: the average initial fresh weight (5 mg) of the postlarval pink shrimp used was one-half that of F74A. Since other experimental conditions were little different between the two experiments, it was probably this factor that led to the reduced survival observed in F74B. Three feeding treatments were applied: wheat bran fermented by Myrothecium sp. SC 87 (product washed and refrigerated); unfermented, unground wheat bran; and a no-feed control. Feeding levels were the same as those of F74A (5 gm/day on a dry weight basis), and experiment duration (60 days) was approximately the same as that of F74A (58days). The fungal material came from Microferm runs 27 and 28 (Table 4), the products of which were very similar (in N% and Δ CP%) to those of the Microferm runs (22 and 25) which yielded the Myrothecium - feed treatment of F74A.

The means of all treatment replicates were significantly different from one another in F74B. The AFFW's and total yields of the unfermented bran replicates of F74B were similar to those of F74A (Fig. 9), and the same was true of the no-feed controls; in both cases, total yields were slightly lower in F74B. The most marked difference between F74A and F74B was the performance of the Myrothecium feed. The AFFW of the higher of the two replicates of the refrigerated feed (F74B) was higher by a factor of 2.4 than that of the higher replicate of the dried feed (F74A). It was also lower than the higher AFFW of the unfermented bran in F74B by a factor of only 0.8. Similar figures applied to comparisons among the same treatments with regard to total yield. These results represented a marked improvement over the dried feed with respect to comparison on a higher-replicate basis to the unfermented bran treatments; the dried-feed AFFW of F74A was smaller by a factor of 0.45 than the AFFW of unfermented bran.

Experiment F74C was begun in August (Table 12). Salinities ranged higher and temperatures lower than in F74A and F74B, but the major differences in experimental conditions were that a different species of shrimp was used (white shrimp, Penaeus setiferus) and average initial fresh weight was much larger (value with 95% confidence interval = 3.27 ± 0.21 gm). For this reason, average fresh weight gain (AFWG) and total fresh weight gain (TFWG) were compared among treatments. Also, stocking density was lower on the basis of number of individuals per m^2 (5), but much higher on the basis of fresh weight per m^2 (8.2gm, as compared to 0.1gm for F74A). Feeding level was 2.5 gm per day on a dry weight basis (approximately 15% of body weight at the beginning of the experiment).

Feeding treatments again included unfermented bran, which for this experiment was ground through a 20-mesh Wiley mill screen. Rather than a no-feed control, an

rcial fertilizer was added at a rate that inorganic nitrogen control was used: a con

of the most nitrogenous of the other made nitrogen addition equivalent to th feeding treatments. Two fungal-fermented feeds were tested, one produced by Pestalotia sp. SC 38 (Microferm runs 31 and 32) and Curvularia sp. SC 86 (Microferm run 29). In both cases, N% and ΔCP% were lower than had been the case with the previously tested Trichoderma and Myrothecium feeds (Table 4). Both fungal feeds of F74C were dried and ground through a 20-mesh Wiley mill screen. One combination treatment was used, a 50-50% mixture of Pestalotia sp. SC 38 dried material and unfermented bran.

The highest AFWG was that of the higher replicate for unfermented wheat bran (Fig. 10), but it was closely followed by that of the higher of the mixed-treatment replicates (lower by a factor of only 0.8, and not significantly different). The TFWG of the higher of the mixed treatment replicates was higher than that of the higher of the bran replicates. The higher replicate of the unmixed Pestalotia-fermented material had an AFWG which was smaller by a factor of 0.6 (significantly different) than that of the higher of the mixed-treatment replicates. The Curvularia replicates fell in a group of means not significantly different from one another, including the inorganic nitrogen controls, and the higher replicate of the Curvularia feed had an AFWG which was only 1.2 times higher than the higher inorganic nitrogen control. In TFWG, the Curvularia results were virtually the same as those of the inorganic nitrogen controls (Table 12).

Pink shrimp postlarvae were again used in experiment F74D, begun in September (Table 12). The average initial fresh weight (17 mg) was larger than that of F74B, but percent survival was again lower than in F74A, probably due to the combined effects of higher salinity range and lower temperature range. Stocking density was one-half that of F74A and F74B, and feeding level was similarly reduced (2.6 gm/day on a dry weight basis).

As in F74C, an inorganic nitrogen control and a 20-mesh unfermented wheat bran treatment were applied. The commercial flake treatment used in F74A was also applied. The three fungal-fermented bran treatments were all mixtures with 20-mesh unfermented bran. Myrothecium sp. SC 87 material (refrigerated, not dried) was mixed 50-50% with unfermented bran. Trichoderma viride SC 51 material was dried and ground through two mesh sizes, 20 and 60. Each of these two sizes served as a separate feeding treatment, mixed at 57% fungal material to 43% unfermented bran. The mixed Trichoderma feeds were fed at 3 gm/day. The fungal materials were produced in Microferm runs 26 and 34-36 (Table 4). Runs 34-36 were lower in N% and markedly lower in Δ CP% than previous runs with the same fungi. In addition, the refrigerated Myrothecium material was accidentally frozen prior to the beginning of F74D, and by the end of the experiment, it had suffered noticeable spoilage.

Because survival was markedly different among treatments and even among replicates, and because there was an apparent marked effect of survival upon AFFW (Table 12), only those replicates with greater than 80% survival were compared by ANOVA and SNK (Fig. 11). As in F74A, the commercial flake replicates were both higher in AFFW than those of the unfermented bran treatment; in F74D, the higher flake replicate was 1.5 times larger in AFFW and total yield than the one bran replicate (and significantly different from it). The unfermented bran AFFW and total yield, however, were virtually identical to those of the one Myrothecium + bran replicate with survival greater than 80%. The higher Trichoderma + bran treatment replicates with survival greater than 80% gave lower AFFW (though significant difference was not detected) and poorer feed efficiencies (by a factor of 1.4) than the higher-survival Myrothecium + bran replicate. There was no apparent difference between the two Trichoderma + bran treatments with different particle sizes.

10. Culture screening on delignified bagasse and straw

Background.

Plant waste materials of minimal acquisition cost are, of course, the eventual target of our feed-development program. The agricultural wastes of least value are those which do not readily serve as productive feeds for terrestrial farm animals, i.e., those which are high in lignified cellulose (Pigden and Heany, 1969). The lignin portion of these materials acts on a molecular level as a physical and/or chemical barrier to cellulose digestion, and thus devalues the materials as feed for ruminants. Bagasse is a good example of an agricultural waste material of this type. It is the remains of the sugar cane stalk after crushing to remove sucrose, and on a dry weight basis, consists of 71% cellulose + hemicellulose, 20% lignin, and 2% silica (Srinivasan and Han, 1969). Materials with lignin and silica contents as high as these serve largely as inert fillers or roughage in feeding of ruminants (Van Soest, 1969). Wheat straw is also one of the lowest quality forages; like bagasse, it is very low in nitrogen content (0.4%), and addition of urea in itself has no effect on its digestibility for ruminants (Pigden and Heany, 1969).

Wheat straw and bagasse, then, are two typical lignocellulosic, low-value agricultural by-products. In addition, both are easily obtainable in South Florida, and bagasse is readily available in the warm tropical countries where shrimp mariculture development is taking place (Gross, 1973; Webber, 1975). Therefore, these two materials were chosen as substrates for a fungal culture screen.

The results of our earlier culture screen on bagasse (Section 6) had demonstrated the marked resistance to fungal decomposition of this material. Other workers had shown a similar response using cellulolytic fungi and bacteria (Chahal et al., 1969; Srinivasan and Han, 1969). As stated above, wheat straw is degraded only to a small extent by rumen microorganisms. However, in the case of both materials, physical and chemical treatment of the materials which alters the lignin-cellulose physical/chemical relationship can greatly improve their degradability (Callihan and Dunlap, 1971; Toyama and Ogawa, 1972; Wilson and Pigden, 1964). Therefore, we applied a delignification (this word is used here to mean alteration of the lignin-cellulose relationship, rather than complete removal of lignin) treatment to the bagasse and straw which was to be used in the culture screen.

Materials and methods.

The treatment was a "dry" method and involved a combination of conditions used in several other delignification studies (Ghose and King, 1963; Han and Srinivasan, 1968; Pew and Weyna, 1962; Toyama and Ogawa, 1972; Wilson and Pigden, 1964). The oven-dried (55°C) materials were ground through a 20-mesh Wiley mill screen, and autoclaved (120°C) for one hour after thorough mixing of a 25% (w/w) suspension of the lignocellulosic materials (200 gms. each were used) in an 8.75% (w/v) NaOH solution in deionized water.(This combina n is closer to a wet solid than a liquid suspension.) When cool, the materials were ed with tapwater over a 245 μm nylon filter cloth until the liquid passing the fil became clear (12 liters were required for each of the materials). The materials w then resuspended in 800 mls of 5% acetic acid, stirred for 60 seconds, and washed h tapwater until constant pH of the resuspended materials was reached (this requi about 10 liters in each case; final pH: straw, 5.4; bagasse, 5.2). In the case both bagasse and straw, the delignification and washing resulted in a 62% loss of ginal dry weight. This was in part due to the loss of finely divided solid materia rough the coarse filter cloth during washing. The materials were then oven-drie t 55°C and ground through a 60-mesh Wiley mill screen.

Due to constraints of time, funds, and equipm availability, it was deemed desirable to use a different culture-screening method m the earlier bagasse screen. It was decided to try an experimental plan insp l by the agar-plate growth measurement methodology developed by Pirt (1966 67), Trinci (1969, 1971), and Morrison and Righelato (1974), and the methods of gal biomass measurement by direct observation of Warnock (1971) and Nagel-deB (1971). Their methods were adapted to yield indices of degree and rate of abi to degrade and convert the bagasse and straw substrates.

The fungi chosen for screening were selected for one or more of the following reasons: high frequency of occurrence on bagasse and/or straw before or during the shrimp-feeding experiment described in Section 5; description in the scientific literature (Sections 3 and 4) as highly cellulolytic and/or of high potential or indicated nutritive value; high nitrogen content produced in wheat bran fermentation or relatively high nitrogen content produced in bagasse fermentation during earlier culture screening (Section 6); isolation from estuarine or maritime cellulosic substrates and availability in our culture collection. Many of the fungi chosen were not taken all the way through the culture-screening procedure. In some cases, this was due to poor growth on the inoculum or test media, but in others, time constraints were the cause.

The experimental procedure was as follows. All cultures to be tested were inoculated onto fresh plates of cornmeal agar (CMA) made up with 15 o/oo seawater. These and all following plates were sealed with masking tape and incubated in darkness at 25°C; as soon as ample vigorous growth was produced on these CMA plates by each fungus, transfer was effected onto plates of 1/4-strength CMA with additional agar to bring it to 2% (1/4 CMA +A). These 1/4 CMA +A plates were of uniform agar media depth (20 ml/plate, 10 cm diameter plates) and were prepared at the same time and using the same batch of 15 o/oo seawater as were all the following plates. The transfer onto 1/4 CMA +A and all following transfers were done uniformly as follows: a sterile #1 cork borer (5 mm diameter) was used to remove a cylinder of agar from the plate to be inoculated; a cylinder was then taken from 5 mm behind the growing edge of the inoculum plate, inverted, and placed in the hole on the plate to be inoculated. The passage across 1/4 CMA + A plates was intended to deplete inocula of excessive endogenous reserves. The fungi were permitted to grow on these until they had reached the edges of the plates or until growth slowed. They were then inoculated onto plates of agar media with incorporated delignified material. These

media ... constituted of ... 3.0% delignified material, 0.05% NH_4NO_3, 0.02% ...

ate of KH_2PO_4, 0.01% yeast extract, 2.0% agar, in 15 o/oo seawater. One

epared delignified straw medium (DSA) and one of delignified bagasse (DBA) was

ates or for each test fungus. Again, these were allowed to grow to the edges of the

fungi until growth slowed. In the case of both 1/4 CMA, PA, DSA, and DBA, s

grew very poorly or not at all and were eliminated from further testing.

ed to From these inoculum plates of delignified media, the fungi were transf

DBA. the culture screen plates. Six media were used, two of which were DSA an

DSA Corresponding plates of media with non-delignified materials were prepared

... and NDBA). Two plates of control media were prepared for each test fungus

water), and one of plain agar CMA (only Difco cornmeal agar added to the 15 o/oo

lia except bagasse or straw. medium (PA) having all the ingredients of the test

ed in the centers of culture- Three cylinders from the inoculum plates were juxta

... from their corresponding ... screen plates. The bagasse and straw received ...

inoculum plates. The PA plates received 2 DSA and one DBA cylinder. The CMA plates received 2 DBA and one DSA cylinder. This rather large inoculum including a portion of the substrate to be fermented was intended to be analogous to the procedures of the fermentation process to be derived from the culture screen results.

Each test plate was read once daily after inoculation. Colony radial growth extent and rate (mm/hr) were read for three radii on each plate. Each of the radii bisected a sector which contained one of the inoculum cylinders. Plates were read until growth of 2 of the 3 sectors reached the edge of the plate, or until growth rate of the 3 sectors slowed by a total of 0.1 mm/hr over a 24-hour period. When this occurred, the plates were first stained with trypan blue in lactophenol, then flooded with a killing and fixing agent (FAA: ethyl alcohol (95%) - 50%, glacial acetic acid - 5%, formaldehyde (40%) - 10%, water - 35% (Sass, 1958)).

The stained and fixed plates were used to make a determination of biomass of fungus produced. A cylinder of agar was removed from 5 mm behind the leading edge of each of the 3 sectors on each plate. A 2 mm thick slice was taken from the top of each cylinder and placed on a microscope slide. An eyepiece with photographic reticle (Wild) having a transecting line was used to measure hyphal density. The eyepiece line was positioned so that it was perpendicular to the majority of hyphae in the field, and all surface hyphae crossing the line were then counted and recorded as hyphae per mm. Cover slips were then placed on the slices and the diameters of 5 randomly selected hyphae from each of the three sectors were measured. The 15 hyphal diameters and 3 densities were averaged to yield values of each for each of the test plates for each fungus.

Using these two measurements, an indicator was calculated of the fungal biomass produced, simply by multiplying mean hyphal density by mean hyphal diameter for both control (PA) and test media, and subtracting the value for PA from that of each of the test media. This is given as biomass index (BI) in Table 14 for those few fungi for which analysis was completed, alongside an indicator of potential utility in the fermentation process to be developed (UI. = BI x maximum growth rate). Growth extent at earl stationary phase is also given in Table 14, but not included in the calculation of the idices (although it might logically have been) for this was the least accurately deter ned of the growth parameters measured.

Results.

Of the 15 fungi r which complete analyses were conducted (Table 14), the 2 species of Drechslera SC 14 and 134) and Chaetomium sp. SC 6 gave the highest BI's on DSA (728, 658, an 578 respectively), and Chaetomium sp. SC 6 gave by far the highest BI (629) on DS . Surprisingly, only 4 of the 15 fungi produced higher BI on DSA than on NDSA. C these, marked increases were shown by Chaetomium sp. SC 6, Pycnoporus sanguineus SC 125, and Trichoderma viride SC 51. This is likely to have

Table 14. Results of a culture screen designed to find strains of fungi capable of efficiently converting "delignified" (alkali-treated) wheat straw and bagasse into fungal biomass.

FUNGI[a]	SUBSTRATE[b]	MAX. GROWTH RATE[c]	GROWTH EXTENT CLASS[d]	MEAN HYPHAL DENSITY[e]	MEAN HYPHAL DIAM.[f]	BI[g]	UI[h]
	PA	0.17	1	9.0	2.1	--	--
	CMA	0.34	3	58.0	3.4	197	67
SC 6	NDS	0.40	3	74.5	4.1	305	122
	DS	0.40	3	121.9	5.4	658	263
	NDB	0.34	2	36.6	2.7	98	33
	DL	0.41	3*	91.3	6.9	629	257
	PA	0.29	3	11.4	2.1	--	--
	CMA	0.37	3*	58.3	2.6	128	47
SC 8	NDS	0.35	3*	42.6	2.8	95	33
	DS	0.33	3	18.6	1.5	4	1
	NDB	0.31	3*	25.2	1.8	21	7
	DB	0.31	3*	12.6	2.0	1	0
	PA	0.31	3*	9.3	3.1	--	--
	CMA	0.35	3	70.9	4.1	262	92
SC 14	NDS	0.31	3	305.1	4.2	1253	388
	DS	0.33	3	302.7	2.5	728	240
	NDB	0.37	3*	36.6	4.5	136	50
	DB	0.30	2	87.1	3.6	284	85
	PA	0.25	1	23.4	2.2	--	--
	CMA	0.36	3	78.1	3.4	214	77
SC 20	NDS	0.37	2	45.6	3.3	99	37
	DS	0.28	3*	43.2	2.1	39	11
	NDB	0.34	2	22.8	3.4	26	9
	DB	0.30	3*	30.6	2.3	19	6

Table 14. (continued)

FUNGI[a]	SUBSTRATE[b]	MAX. GROWTH RATE[c]	GROWTH EXTENT CLASS[d]	MEAN HYPHAL DENSITY[e]	MEAN HYPHAL DIAM.[f]	BI[g]	UI[h]
	PA	0.61	3*	8.7	4.5	--	--
	CMA	0.83	3*	74.5	5.2	348	289
SC 51	NDS	0.79	3*	58.9	4.9	249	197
	DS	0.80	3*	106.3	3.9	375	300
	NDB	0.71	3*	20.4	3.7	36	26
	DB	0.75	3*	82.3	4.3	315	236
	PA	0.34	2	5.7	3.7	--	--
	CMA	0.32	3*	27.0	3.5	73	23
SC 77	NDS	0.34	1	96.7	4.2	385	131
	DS	0.36	1	53.1	4.3	207	75
	NDB	0.35	3*	35.4	4.5	138	48
	DB	0.30	3*	33.6	3.5	97	29
	PA	0.25	2	5.3	2.4	--	--
	CMA	0.50	3*	18.6	4.8	77	38
SC 90	NDS	0.44	2	30.0	3.7	98	43
	DS	0.35	3*	20.4	2.5	38	13
	NDB	0.31	3	6.3	3.5	9	3
	DB	0.32	3*	13.5	3.3	32	10
	PA	0.37	3*	58.9	4.9	--	--
	CMA	0.39	3*	120.1	4.2	216	84
SC 98	NDS	0.43	3*	77.5	5.1	107	46
	DS	0.41	3*	97.3	3.5	52	21
	NDB	0.37	3*	93.7	3.3	21	8
	DB	0.38	3*	141.2	3.2	163	62

Table 14. (continued)

FUNGI[a]	SUBSTRATE[b]	MAX. GROWTH RATE[c]	GROWTH EXTENT CLASS[d]	MEAN HYPHAL DENSITY[e]	MEAN HYPHAL DIAM.[f]	BI[g]	UI[h]
	PA	0.18	1	24.9	1.6	--	--
	CMA	0.24	2	38.7	2.3	49	12
SC 100	NDS	0.30	2	91.9	2.1	153	46
	DS	Lost	1	15.6	1.5	--	--
	NDB	0.24	3*	33.6	1.7	17	4
	DB	0.14	1	20.7	1.4	-11	--
	PA	0.06	0	9.0	2.3	--	--
	CMA	0.19	2	39.9	2.7	87	17
SC 101	NDS	0.45	3*	75.7	4.9	350	158
	DS	0.43	3	51.6	4.5	212	91
	NDB	0.41	3*	27.0	5.4	125	51
	DB	0.45	3*	54.7	3.9	193	87
	PA	0.19	3	17.4	2.1	--	--
	CMA	0.19	0	91.3	1.8	128	24
SC 114	NDS	0.25	3*	94.6	1.9	143	36
	DS	0.22	1	104.5	2.0	172	38
	NDB	0.21	3*	22.8	3.4	41	9
	DB	0.19	3	69.7	3.7	221	42
	PA	0.28	3*	9.9	5.7	--	--
	CMA	0.13	2	60.7	4.0	186	24
SC 122	NDS	0.30	2	111.7	4.6	457	137
	DS	0.28	3*	48.6	3.8	128	36
	NDB	0.35	2	36.3	4.7	114	40
	DB	0.29	3*	28.8	3.5	44	13

Table 14. (continued)

FUNGI[a]	SUBSTRATE[b]	MAX. GROWTH RATE[c]	GROWTH EXTENT CLASS[d]	MEAN HYPHAL DENSITY[e]	MEAN HYPHAL DIAM.[f]	BI[g]	UI[h]
	PA	0.22	2	70.2	3.2	--	--
	CMA	0.26	2	303.9	2.8	626	163
SC 125	NDS	0.25	3*	193.4	2.5	259	65
	DS	0.25	3*	217.4	2.9	406	101
	NDB	0.21	2	91.0	3.5	94	20
	DB	0.24	3*	164.6	2.5	187	45
	PA	0.54	3*	25.5	2.1	--	--
	CMA	0.40	3*	69.7	2.6	128	51
SC 133	NDS	0.35	3*	94.9	2.2	155	54
	DS	0.38	3*	80.5	2.0	107	41
	NDB	0.36	3*	34.8	1.9	13	5
	DB	0.38	3*	54.7	1.3	18	7
	PA	0.20	2	9.9	2.6	--	--
	CMA	0.20	1	73.0	4.5	303	61
SC 134	NDS	0.31	2	158.6	6.3	973	302
	DS	0.30	2	133.3	4.6	587	176
	NDB	0.26	3*	7.8	2.3	-8	--
	DB	0.25	2	45.1	3.7	141	35

[a] Fungal strains listed by accession (culture) number; SC 6--*Chaetomium* sp.; SC 8--*Scopulariopsis*(?) sp.; SC 14--*Drechslera* sp.; SC 20--*Zythia* sp.; SC 51--*Trichoderma viride*; SC 77--*Dendryphiella salina*; SC 90--*Aspergillus niger*; SC 98--*Pestalotia* sp.; SC 100--*Sporormia* sp.; SC 101--*Chaetomium* sp.; SC 114--*Aspergillus terreus*; SC 122--*Alternaria longissima*; SC 125--*Pycnoporus sanguineus*; SC 133--*Schizophyllum alneus*; SC 134--*Drechslera* sp.

Table 14. (continued)

[b] PA = plain agar (control); CMA = cornmeal agar (Difco); NDS = non-delignified straw; DS = delignified straw; NDB = non-delignified bagasse; DB = delignified bagasse. All media 2.0% agar (Difco) (except CMA), 0.25% NH_4NO_3, 0.09% KH_2PO_4, 0.01% yeast extract (Difco), 2.9% test substrate (except CMA), in 15 o/oo seawater.

[c] Maximum growth (diametric extension) rate observed over a 24-hour period, in mm/hr.

[d] 0 = 0-10 mm; 1 = 10-20 mm; 2 = 20-30 mm; 3 = 30-40 mm; 3* = rate of growth not declining when growing edge had reached plate's edge. Plates were flooded with fixative (FAA) when growth rate declined by an average of 0.03 mm/hr or more over a 24-hour period.

[e] Across a 1 mm-long transect made perpendicular to the growing hyphae 5 mm from the growing edge.

[f] In μm, average of fifteen randomly-selected hyphae.

[g] BI = biomass index = (mean hyphal diameter (MHDI) on test substrate x mean hyphal density (MHDE) on test substrate) - (MHDI on plain agar x MHDE on plain agar).

[h] UI = apparent utility index = BI x maximum growth rate.

been an effect of excessive alkali treatment and thorough washing having removed soluble nutrients, including otherwise available hemicellulose (Wilson and Pigden, 1964; Garcia-Martinez et al., 1974) which may have positive effects on cellulolysis and growth (Basu and Ghose, 1960; Nilsson, 1974).

Of the 11 fungi which produced higher BI on NDSA than on DSA, the highest values and most marked differences were shown by the Drechslera spp. (SC 14 and 134) and Alternaria longissima SC 122. To the contrary, 11 of the 15 fungi produced higher BI on DBA than on NDBA, highest values and most marked differences being shown by Chaetomium sp. SC 6, Trichoderma viride SC 51, and Aspergillus terreus SC 114. Of the four which did not, only Alternaria longissima SC 122 produced a markedly higher BI on NDBA than on DBA, and the other three had low BI's on both media. Thirteen of the 15 fungi had higher BI values on the straw agars than on the corresponding bagasse agars. Of the two which were otherwise, Pestalotia sp. SC 98 had highest BI on CMA, and Aspergillus terreus SC 114 was the only test fungus with highest BI on DBA.

Maximal radial growth rates were much less evidently affected by the test substrates than hyphal diameter and especially hyphal density, so that within a fungal test group, UI values reflect BI values. However, because of its very rapid growth rate (approximately 0.75 mm/hr, 24-hr mean, on all media except PA), Trichoderma viride SC 51 produced the highest UI on DSA (300), followed by Chaetomium sp. SC 6: 263, and Drechslera sp. SC 14: 240, and was second only to Chaetomium sp. SC 6 on DBA (SC 6: 257, SC 51: 236; next highest was 87 for Chaetomium sp. SC 101). Highest UI on NDSA was 388 (Drechslera sp. SC 14) followed by Drechslera sp. SC 134 at 302, and the next highest was Chaetomium sp. SC 101 at 158.

The values of UI and BI for CMA relative to these values for the other test substrates varied among the fungi tested. Some fungi exhibited highest values on CMA (e.g. Pycnoporus sanguineus SC 125 and Pestalotia sp. SC 98), while others exhibited values on CMA as low or lower than those on NDB, which was, in general, the poorest substrate (e.g. Dendryphiella salina SC 77 and Chaetomium sp. SC 101).

DISCUSSION

11. Fungal fermentation of wheat bran

Wheat bran was not chosen as a substrate for our initial fungal fermentations because of its potential for scaled-up commercial production of feed. Its cost is substantially higher (5 times or more) than that of the nearly entirely lignocellulosic agricultural by-products since its relatively high soluble carbohydrate (53%) and protein (17%) content give it value as a feed fraction and bakery product. Rather, we chose it because, of the four plant materials tried as artificial detrital materials by Caillouet et al. (1975b; Section 5), it was the only one which : (1) supported marked growth of shrimp when fed without treatment (Caillouet et al., 1973, 1975a, b); and, more importantly, (2) permitted marked growth of fungi in submerged fermentation.

We chose wheat bran in order to determine on a preliminary basis: (1) whether the concept of feeding of fungal fermented materials was a practicable one; and (2 whether the yield of shrimp producible with untreated wheat bran could be improved by fermentation, beyond the size satisfactory for a commercial bait-fishery concern.

Our culture screen results indicate the extent to which the protein content o wheat bran can be changed by fungal fermentation (under the specific condition imposed) (Tables 2 and 3). Conditions of the two screens differed in several respect (Section 6), but perhaps most importantly in that phosphate concentration an especially inorganic nitrogen concentration were markedly higher in the secon screen. Probably as a consequence, the percentage of nitrogen in the product wa pushed higher in the second screen (Fig. 1); the mutability of nitrogen content c fungal mycelium as influenced by the C/N ratio of the growth medium is well know (e.g. Cowling, 1970; Lilly, 1965; Section 3). It is probably fair to suggest that th higher nitrogen contents reflect increases in true protein content, for Levi and Cowling (1969) and Graham (1971) found that decrease in the C/N ratio of growth medium leads to increase in non-nucleic nitrogen of mycelium, but does not result in an increase of mycelial glucosamine or ammonia.

There was a lesser effect on the percentage change in crude protein; the greatest fraction of those fungi tested reduced the crude protein by [illegible] in both screens, though the second screen produced fewer high losses and more high gains. The difference in extent to which the nitrogen content of the mycelium of a given fungus is mutable is shown by the three strains of fungi which were tested in both screens; one (Lulworthia sp. SC 73) produced nearly identical results in both screens, while the other two (Trichoderma viride SC 51 and Pestalotia sp. SC 38) showed increases in percentage of nitrogen of 8.3 and 12.5 respectively. These increases may also have been partially due to increased phosphate concentration. The PO_4 concentration used in the first culture screen (0.006%) is likely to have been limiting (Child et al., 1973), unless PO_4 present in the wheat bran could alleviate the shortage.

Comparison of our wheat bran culture screen results with the results of other investigators who have attempted protein production by fermentation of substrates of high starch content is difficult for the following reasons. (1) In some cases, results are incompletely presented, due to the proprietary nature of the research. (2) Entirely soluble media were used, rather than solubles + particulate suspension—thus separation of mycelium from unconverted material was possible. (3) Determination of mycelium production by nitrogen analysis of the fermented wheat bran products was not possible because the original protein content of wheat bran is considerable. (4) Differences of opinion exist among investigators with regard to the best means of determining protein content of fungal mycelia (e.g. Christias et al., 1975; Coleman, 1972; Herbert et al., 1971; Solomons, 1973) resulting in the use of several methods (Lowry, Biuret, α-amino nitrogen, total amino acids, total nitrogen); conversion of figures derived by one method to those derived by another are impossible. (5) Differing methods of presentation of results have been used (biomass/carbohydrate utilized, protein/carbohydrate utilized, protein/volume of fermentation liquid, etc.). (6) Although wheat bran is a common growth medium used in the production of fungal enzymes (Codner, 1969; Ghandi et al., 1974; Underhofler et al., 1947; Wang et al., 1974) we know of only one very recent report of its use for the production of fungal biomass (Von Hofsten, 1974). With regard to item (4), it is perhaps best in our particular case to use total nitrogen x 6.25, since the major part of the error in calculating true protein from total nitrogen lies in the contribution of chitin nitrogen. At least a portion of the chitin may be nutritively valuable to shrimp (Section 4).

Results of several studies of protein production by fungal fermentation of largely-starch or simple-sugar substrates are given in Table 15. When substrates such as these are utilized for production of fungal biomass, and only the resultant mycelium is collected, the crude protein content of the products can range quite high,

(to 74%). If, however, the solid undigested remainder of the original sub
included in the fermentation product, the upper end of the range is lower (38
non-nitrogenous, unfermented plant structural materials (some combir
cellulose, hemicellulose, lignin, pectin, residual starch) remain. The resul
wheat bran fermentations, the products of which include undigested bran fi
much the same in terms of range of crude protein content as those usi
substrates in particulate suspension. The values for protein production giver
15 are dependent upon concentration (w/v) of substrate used in the ferment
upon ability of the fermenting microorganism(s) to efficiently convert
microbial biomass at those concentrations. Thus, they are related to
production efficiency (biomass produced/unit volume of fermentation m
time), though the figures in Table 15 do not include growth-rate information.

The wheat bran values (and probably the value for most of t

Table 15. Results of studies of fungal protein production from amyloid and/or simple-sugar substrates comparable to the present wheat bran fermentation results.[a]

Reference	Substrate[b]	#Strains	Crude Protein %[c]		Protein Yield[d]	
			Low	High	Low	High
Solomons, 1973	NR[e]	"hundreds"	NR	74	NR	NR
Falanghe *et al.*, 1964	soybean whey, L,9	4	33	64	0.5	4.6(+1.3)
Graham, 1971	glucose, L, NA[f]	3	35	62	0.5	2.9
Christias *et al.*, 1975	glucose, L, NA	4	39	58	NR	NR
Bednarski *et al.*, 1971	milk whey, L,0	4	16	57	3.9	23.3[i]
Litchfield, 1968[h]	several, NA	33	3	54	NR	NR
Reade *et al.*, 1974	cassava, L, 0.4	"extensive screening"	NR	48	NR	NR
Brook *et al.*, 1969	cassava, S[g], 0.4	28	16	42	1.0	2.8
Shukla & Dutta, 1967	molasses, L, 4.6	1	25	38	2.5	8.7(+5.9)
Gray, *et al.*, 1964	glucose, L, NA	175	13	31	1.2	3.4
Gray & Abou-El-Seoud, 1966a	sweet potato, S,7	6	15	38	1.0	5.9(+0.9)
Culture screen II	wheat bran, S,17	25	21	34	6.7	12.8(+4.3)
Chahal & Cheema, 1971, 1972	rice, S,7	20	21	33	0.4	2.5(+1.5)
Microferm[j]	wheat bran, S,17	4	25	32	2.5	10.6(+2.1)
BFIE[k]	wheat bran, S,17	4	22	31	2.7	19.0 (+5.4)
Reade *et al.*, 1972	barley, S,11	23	8	31	NR	2.8 (+0.6)
Gray & Karve, 1967	rice, S,7	14	24	30	1.8	4.6 (+3.0)
Gray & Abou-El-Seoud, 1966c	sugar beets, S,3.4	9	22	29	1.2	4.2 (+3.1)
Culture screen I	wheat bran, S,17	27	15	29	4.5	9.9 (+0.6)
Gray & Abou-El-Seoud, 1966b	cassava, S,2.8	5	13	20	1.2	3.0 (+2.2)

Table 15 (continued)

[a] Listed in decreasing order of maximal crude protein content; fermentations of entirely liquid media are listed first, followed by those of liquid suspensions of particles.

[b] Listed with an indication of medium type (L = entirely liquid, S = suspension of particles), and the initial crude protein percent.

[c] Of final dried product.

[d] In mg/ml of medium. Numbers in parentheses indicate change in mg/ml from original.

[e] Not reported or determinable from the reference cited.

[f] Not applicable.

[g] Particulates were removed after fermentation and not included in the protein analysis.

[h] A review paper.

[i] It is not clear whether the mycelial product was washed.

[j] Results of 10.5-liter fermentations; see Table 4.

[k] Results of the bran fermentation improvement experiment; see Tables 11-12 and Figures 8-11.

particulate-suspension fermentations in Table 15) are likely to include protein remaining or converted from original substrate protein in addition to that produced from inorganic nitrogen. Therefore, production of protein over quantities previously present is given in parentheses. Since some of the original protein is undoubtedly lost from the solid phase during the fermentations (Section 8), and this was not measured, the actual protein production values are not known. The highest values for fungal protein production over that originally present in wheat bran are as high as the highest of the other values of protein production given in Table 15, except for those of Bednarski et al. (1971). Higher values not yet reported may well have been achieved in the recent extensive research projects of Reade et al. (1974), Solomons (1975), and Worgan (report due for publication in J. Sci. Fd. Agric.).

The protein production figures of Bednarski et al. in Table 15 are an order of magnitude higher than most of the others given. Prefermentation of milk whey by bacteria, followed by mold fermentation, is reported to have been the reason for these high values, but confirmation of these results is needed: the concentration of easily fermentable sugars and conversion efficiencies necessary to have achieved these results would have to have been quite high; the production results of El-Akher et al. (1974), working with yeast fermentations of milk whey, do not approach those of Bednarski et al., washing of the fermentation product is not reported by Bednarski et al.

12. Effect of simultaneous modification of fermentation conditions

Comparison of the crude protein contents and especially protein production values for the four phases of the present project shown in Table 15 give evidence of the extent to which alteration of fermentation conditions can improve production performance. The high crude protein contents reported by Falanghe et al. (1964), Graham (1971), and Solomons (1973) were also achieved by this means. Graham (1971) and Pinto (1963) among others had demonstrated the remarkable extent to which inorganic nitrogen source and nitrogen content of fermentation substrate could affect protein content and amino acid balance of fungal biomass. Pinto's work clearly demonstrated that use of $(NH_4)_2SO_4$ could have a positive influence on content of the sulphydryl amino acids (one of the most common deficiencies of fungal protein--Chiao and Peterson (1953); Litchfield (1968); Rhodes et al. (1961); Thatcher (1954)). Other workers (Hueck and Hazeu, 1969; Meyers 1968; Sguros et al., 1973) had concluded that NH_4NO_3 could serve as well as or better than several other nitrogen sources in providing for good growth of fungi. Therefore, we examined the effects of two levels of NH_4NO_3 concentration and one level of $(NH_4)_2SO_4$ in the fermentation medium on the final nitrogen percentage and crude protein increment produced in fungal-fermented wheat bran. Gray et al. (1963) and Jones and Irvine (1971) had shown that salinity of the fermentation medium could have marked effects on mycelial and protein yield, so we examined this phenomenon. Finally, we examined the effects of concentration of substrate, which is one of the most direct determinants of volume production efficiency (Section 11), and thereby one of the most important factors in economic evaluation of any developmental fermentation process (Callihan and Dunlap, 1971; Gray, 1971).

The results of our comparison of nitrogen sources with regard to their effectiveness in stimulating protein production (Section 8) reflect the findings of other investigators who have examined the question of optimal nitrogen source for fungal fermentations (Table 16). Interaction is common among the effects of type of nitrogen source and several other variables (type of carbon source, concentration of carbon source, medium pH and medium buffering, other medium constituents, and, especially, species of fungus used). Also, optimal nitrogen source may be different depending upon the goal of the developers of the fermentation process (i.e. whether optimized nitrogen percentage of final product, optimal protein production, or optimal biomass production is desired). It is interesting to note, in the case of two fungal strains in the present study (Lulworthia sp. SC 73 and Myrothecium sp. SC 87),

Table 16. The effectiveness of types of nitrogen source in stimulating fungal production processes.

Reference	Production & Substrate[a]	Fungi	Order of Effectiveness
Sekeri-Pataryas *et al.*, 1973	% CP-carob extract	*Aspergillus niger*	$(NH_4)_2SO_4 > (NH_2)_2CO$
	DW "	"	$(NH_4)_2SO_4 \approx (NH_2)_2CO$
Chahal & Cheema, 1972	% CP & DW-rice	*Penicillium crustosum*	$KNO_3 > (NH_2)_2CO > NH_4CL > NH_4NO_3$
Shukla & Dutta, 1967	DW & CP/vol-molasses	*Rhizopus* sp.	$NH_4CL > (NH_4)_2SO_4 > (NH_2)_2CO$
Graham, 1971	DW & CP/vol-glucose	*Rhizopus oligosporus*	$(NH_4)_2SO_4 > NH_4CL > (NH_2)_2CO > (NH_4)_2HPO_4$
	DW-potato starch, pH 4	"	$(NH_4)_2HPO_4 > (NH_4)_2SO_4$
	DW-potato starch, pH 6	"	$(NH_4)_2SO_4 > (NH_4)_2HPO_4$
	CP/vol-potato starch, pH 4	"	$(NH_4)_2HPO_4 > (NH_4)_2SO_4$
	CP/vol-galactose	"	$(NH_4)_2SO_4 > (NH_4)_2HPO_4$
Pinto, 1963	CP/vol-glucose	*Heterocephalum aurantiacum*	$(NH_2)_2CO > (NH_4)_2SO_4 > NH_4NO_3 > NH_4CL$
	"	*Cladosporium* sp.	$NH_4CL > (NH_2)_2CO > NH_4NO_3 = (NH_4)_2SO_4$
	"	*Spicaria elegans*	$NH_4CL = (NH_4)_2SO_4 > (NH_2)_2CO > NH_4NO_3$
	"	*Linderina pennispora*	$(NH_4)_2SO_4 > (NH_2)_2CO > NH_4CL > NH_4NO_3$
Yusef & Allam, 1967	DW-glucose	*Chaetomium* sp.	$(NH_2)_2CO > NaNO_3 > (NH_4)_2SO_4$
	"	*Pestalotia gracilis*	$NaNO_3 > (NH_2)_2CO > (NH_4)_2SO_4$
	"	*Myrothecium verrucaria*	$NaNO_3 = (NH_2)_2CO > (NH_4)_2SO_4$
Verona *et al.*, 1973	% TAA-glucose	*Aspergillus niger*	$NH_2CN > (NH_2)_2CO > NH_4NO_3 > NaNO_3$
	"	*Penicillium frequentans*	$(NH_2)_2CO > NH_4NO_3 > NH_2CN > NaNO_3$

Table 16. (continued)

Reference	Production & Substrate[a]	Fungi	Order of Effectiveness
Agnihotri, 1964	DW-sucrose	*Aspergillus quadrilineatus*	$NaNO_3 > (NH_2)_2CO > (NH_4)_2SO_4$
	DW-starch	"	$(NH_2)_2CO > NaNO_3 > (NH_4)_2SO_4$
Sguros and Simms, 1963	DW-glucose	*Halosphaeria mediosetigera*	$KNO_2 > KNO_3 > (NH_4)_2CO_3 > NH_4NO_3 > NH_4CL > (NH_4)_2SO_4$
	", buffered, pH 8.2	"	$(NH_4)_2SO_4 = NH_4NO_3 = KNO_2 = NH_4CL > KNO_3 > (NH_4)_2CO_3$
Jones and Irvine, 1971	CP/vol-cellulose	*Corollospora cristata*	$(NH_2)_2CO > KNO_3 > NH_4NO_3 > NH_4CL$
	"	*Dendryphiella salina*	$(NH_2)_2CO > KNO_3 = NH_4NO_3 > NH_4CL$
	"	*Cladosporium herbarum*	$(NH_2)_2CO > NH_4NO_3 > NH_4CL > KNO_3$
	"	*Lulworthia* sp.	$(NH_2)_2CO > KNO_3 = NH_4CL > NH_4NO_3$
Hueck and Hazeu, 1969	radial growth	5 common cellulolytic species	$NH_4NO_3 = NaNO_3 > (NH_2)_2CO > NH_4CL$
	cellulolytic activity	"	$NH_4NO_3 > (NH_2)_2CO > NH_4CL > NaNO_3$
Chahal and Gray, 1968	CP/vol-wood pulp	44 species	$(NH_2)_2CO > KNO_3 > NH_4CL$
El-Kersh *et al*., 1973	cellulase-CM cellulose	*Phoma glomerata*	$NH_4NO_3 = NaNO_3 > NH_4CL > (NH_2)_2CO$
	"	*Rhizoctonia solani*	$NaNO_3 > NH_4NO_3 > (NH_2)_2CO > NH_4CL$
Siu, 1951	cellulolysis	*Aspergillus fumigatus*	$(NH_4)_2SO_4 > NaNO_3 > (NH_2)_2CO$
	"	*Humicola grisea*	$(NH_2)_2CO > (NH_4)_2SO_4 > NaNO_3$
	"	*Aspergillus terreus*	$NaNO_3 > (NH_4)_2SO_4$

[a] % CP = crude protein percentage; DW = dry weight; CP/vol = crude protein per volume of medium; %TAA = percentage of total amino acids.

that although nitrogen source had a marked effect on final percentage of nitrogen in the fermented product, there was no detectable effect upon the absolute change in crude protein, meaning that the deficiencies in production of a highly nitrogenous product were balanced by difference in mycelial biomass produced or original material lost.

Clearly it is of little value to determine optimal nitrogen source for fungal fermentations without examining simultaneously the effects of other potentially interacting variables. However, two generalities which appear upon examination of the references cited in Table 16 are: (1) as Jones and Irvine (1971) point out, when urea, $(NH_2)_2CO$, has been tested, it has often performed as well as or better than other simple nitrogen sources; (2) as Nicholas' review (1965) and Sguros and Simms' (1963) work show, when ammonium salts are to be used, it is advisable to use buffered media. The present study did not include examination of applicability of these generalities to wheat bran fermentations; it is quite possible that higher levels of protein production might have been achieved had urea been used as a nitrogen source, or had better buffered media been used with the ammonium salts used.

Final percentages of nitrogen and crude protein yields of the present experiment appeared to depend more heavily on type of nitrogen source than on C/N ratio except in the case of Leptosphaeria maritima RZ 312 at the highest bran concentration, when the lower C/N gave markedly higher final nitrogen percentage and crude protein increment. That C/N had no effect on final nitrogen percentage of the Lulworthia products was to be expected, since this fungus had shown nearly equivalent results in the two culture screens, which had utilized media with much different C/N ratios (51 in culture screen I and 14 in culture screen II).

The two C/N ratios used in our experiment (14 and 28, considering only ammonium nitrogen--see Section 8; these values fall to about 7.5 and 10 when the bran nitrogen is included) lie in or near the range (5-25) given by Litchfield (1968) as optimal for fungi in general with regard to fungal protein production. The C/N ratio must be interpreted with caution in media including complex substrates such as wheat bran, especially when ammonium and nitrate ions are present; not all of the organic carbon (Von Hofsten and Von Hofsten, 1974) or nitrogen of the wheat bran is available to fungi, and ammonium ion uptake depresses or prevents nitrate ion uptake (Nicholas, 1965), so that the ratio of available carbon to available nitrogen is not known, but is certainly different from the ratio of carbon present to nitrogen present.

Our findings w regard to salinity's effects on protein production are in line with those of Jones d Irvine (1971); in both cases, salinities near 10% the ionic strength of full sea r resulted, in general, in best fungal protein production. It is interesting to note t in both studies, the obligately marine fungi showed the clearest increases in otein production as salinity decreased from 30 o/oo to about 5 o/oo. This general tern was clearer than the one observed with type of nitrogen source (Section 8), here too, salinity had significant interacting effects with the other treatment var es (in five of the eight possible cases, considering both final nitrogen percentage crude protein increment).

Significant eff were detected for the modified concentrations of wheat bran in every tested case ht of eight), and significant interaction was detected in seven of these cases. The y general conclusion which can be reached, and this applies to all three of the trea nt variables examined, is that in experimentation designed to determine optimum nditions for a given developmental fermentation, one must determine where int ependencies exist among conditions which can be varied, and systematically alter em together, as many at a time as is practicable, if one is to discover optimal pr n production levels for given fungal strains. For example, if we had examined th rotein production capacity of Leptosphaeria maritima RZ 312, varying only nitrog source at 2% bran and 30 o/oo salinity, we would have concluded that (NH SO_4 produced optimal performance (Fig. 3). Then, varying salinity at 2% bran with $(NH_4)_2SO_4$, we would conclude that 30 o/oo permitted best performance. Finally, varying bran concentration at 30 o/oo and with $(NH_4)_2SO_4$, we would conclude that bran concentration had little effect, and miss the peak performance at 8% bran with NH_4NO_3 and 5 o/oo salinity!

If apparatus for continuous culture is available, the recently reported method of media optimization of Mateles and Battat (1974) might be adaptable to improvement of fungal fermentations.

13. Feeding of fermented wheat bran to penaeid shrimp

As documented above, wheat bran was not chosen because of its high potential as a substrate for production of artificial detrital foods, but rather largely as a matter of convenience. Wheat bran is not likely to serve well as an artificial detrital material for three basic reasons: (1) it has a relatively high acquisition cost (in 1973, it was selling commercially at approximately 5¢/lb. in Texas (personal communication, Reliance Chemicals Corporation)); by contrast, the cost of bagasse is about 1¢/lb. (Callihan and Dunlap, 1971); (2) wheat bran is not readily available in the Central and northern South American countries where commercial mariculture development is now centered (Webber, 1975); (3) wheat bran is composed of both easily fermentable material (starch and protein) and highly refractile material (native cellulosics) (Caillouet et al., 1975; Von Hofsten and Von Hofsten, 1974). Caillouet et al. give a proximate analysis which shows a 53% soluble-carbohydrate content and 11% "fiber," but the Von Hofstens report a 56% cellulose + hemicellulose content. The difference probably lies in the excessive solubilization of hemicelluloses which takes place in proximate analyses (Von Soest, 1969). In any case, some portion of the cellulosic material is probably potentially available to some fungi, i.e., not so heavily lignified that fungal enzymes cannot degrade it. Yet even this portion is probably protected to some extent by the high starch and protein content of wheat bran, since fungal cellulolysis is often inhibited by the presence of more easily degradable carbon sources (Basu and Ghose, 1960; Bravery, 1968; Hueck and Hazeu, 1969; King and Eggins, 1973; Zeltin, 1970; and especially Tashpulatov and Teslinova, 1974).

Item (3) from the above paragraph has an important bearing on interpretation of the results of our shrimp-feeding tests. Because of the relatively high starch (wheat starch was shown by Forster and Gabbott (1971) to be very effectively assimilated by prawns) and protein content, wheat bran, fed directly to penaeid shrimp with no treatment whatever, will support the growth of shrimp to a size and at a rate satisfactory for a commercial bait fishery (Caillouet et al., 1973, 1975a, b). Performance of wheat bran, in terms of yield of shrimp produced, was often only slightly lower in both Caillouet's experiments and in the present experiments, than commercial (or developmental commercial) pelleted or flake feeds (Glencoe pellets, Purina catfish pellets, Purina Experimental Marine Ration 20). In Caillouet's experiments, yields with wheat bran ranged from 69 to 100% of yields with commercial feeds. Any fermentation of the bran will necessarily reduce the available energy per unit of original starting material (Von Hofsten, 1975), and since much of the lignified cellulose and hemicellulose will not be converted without pretreatment (several reports in Gould, 1969; Rogers and Spino, 1973), this fraction will be higher in the fermented product than in untreated bran. The cellulosic fraction of the bran is no more available as nutritive matter to penaeid shrimp than it is to the fungi. Cellulose has been used in shrimp feeds as an inert filler—e.g. Andrews et al. (1972); Forster and Gabbott (1971) found that calories of even a finely ground purified cellulose were assimilated by prawns to only a small extent. Therefore, on a unit dry weight basis, fermented bran will have a lower quantity of nutritively valuable material and available energy than untreated bran. Since the feeding treatments used in our feeding studies were applied at equivalent rates of dry weight per day, the animals receiving fermented bran were receiving less nutritive material than those receiving untreated bran.

Our 14-liter fermentations of wheat bran (Table 4) were conducted largely for production of material for feeding to shrimp, as was the case in the paper fermentation and chick-feeding study of Crawford et al. (1973); also in parallel with their work, the large-scale fermentations yielded lower quantities of protein per unit weight of original substrate in most cases, than had similar small (flask) scale fermentations, due to greater loss of dry weight with respect to the original. Loss of efficacy in yields of fermentations due to scaling up is common and to be expected in developmental studies of this kind (Gray and Abou-El-Seoud, 1966a; Hishinuma et al., 1972; Solomons, 1969). It is due to subtle differences in preparation and sterilization of media, in preparation of inoculum, and in oxygenation and other transport phenomena (Solomons, 1972). Systematic analysis of the variables involved in the 14-liter fermentations was not conducted and more than one variable was often changed from run to run. Therefore, reasons for scale-up problems were not pinpointed. It is important to note that where crude protein produced was less than in small-scale fermentations, and more of the original weight was lost to respired gas on solubilization, the remaining product would contain a greater fraction of the cellulosic material not nutritively valuable to penaeid shrimp.

The results of our feeding experiments exhibit the lack of replicability among feeding treatments which is to be expected when experiments are conducted in outdoor facilities under semi-natural conditions. Under these conditions, interacting uncontrolled variables such as development of microbial and meiofaunal populations, weather effects, development of parasite and pathogen problems, etc., are not prevented from imposing marked and differing effects on shrimp growth. Problems of this sort are discernible in the results presented for other studies of this kind, for example, by Caillouet et al. (1973) and Parker and Holcomb (1974), and Zein-Eldin and Meyers (1974) encountered and discuss the problem. Until these natural variable factors can be better controlled, it is the higher levels of production achieved which should be noted, for these represent indications of levels attainable when some measure of control over experimental variables is accomplished; for this reason, we did not combine means for replications of treatments in our feeding experiments.

The range of responses to fungal-fermented feeds in our experiments ranged from very poor yields of shrimp due to a possible effect of toxicity, to slightly higher yields than unfermented bran. The yields using cold-stored Trichoderma viride material seemed to indicate toxicity, for they were lower than yields when no feed was supplied, due to heavier mortality. Cases of toxicity have been reported for T. viride (e.g. Mirocha and Christensen, 1974), but so have cases of good performance of T. viride mycelium as feed material (e.g. Church et al., 1972). Improper refrigeration certainly could have been the factor responsible for the poor performance of this particular feed; when the T. viride material was dried, excessive mortalities did not occur.

The dried T. viride product gave poor shrimp yields relative to unfermented bran (feed efficiency $\geq$ 20) except when combined with commercial flake material (90% + 10%, respectively), but best feed efficiency was still only 0.7 that of unfermented bran. Two other dried fermented products, those of Myrothecium sp. and Curvularia sp., also gave feed efficiencies greater than 20. It is quite possible that the drying process used was at least in part responsible for the low nutritive capacity of these products. Bauer-Staeb and Bouvard (1973) found that heating and drying had very marked negative effects on the availability of yeast-cell proteins to digestive enzymes, and Spicer (1973) points out that excessive heat processing can reduce the availability of amino acids of fungal protein. Bressani (1968) and Kihlberg (1972) discuss the problem of processing of single-cell protein, they point out that treatments such as heating and drying can impair nutritive capacity by reduction of amino acid availability and/or loss of functional quality (e.g. water absorption capacity). Bärlocher and Kendrick (1973) found that detritivorous amphipods would not accept dried fungal mycelium, though fresh mycelium was avidly eaten.

After harvest of F74C, which utilized large and easily dissected shrimp, contents of the foregut and hindgut were examined. It was observed that the fibrous bran flakes were passing through the shrimp largely undigested, and that fungal material which had been dried onto the flakes was also passing through undigested (the percentage which went undigested was not determined). When the Myrothecium product was refrigerated rather than dried (and spoilage did not take place), a marked improvement in performance in terms of maximal shrimp yield was observed (Table 11, treatments 9 and 10). Drying of the products is, of course, much more desirable than cold storage in terms of development of an economically desirable product, and further experimentation may demonstrate that other methods of drying, or drying of some other developmental fungal feeds, do not detract from nutritive capacity. Drying has improved the nutritive value of single-cell protein in other cases (Kihlberg, 1972; Mogren et al., 1973). See also Regnault et al. (1975) in this regard.

The refrigerated Myrothecium product (F74B) gave a maximal shrimp yield and average final fresh weight per shrimp which were only 0.8 those of the maximal yield of untreated bran of F74B. If we consider the question of relative quantities of nutritive value between the fermented and unfermented bran in this case, using conservative calculation of conversion of wheat bran to mycelial biomass, we find that unfermented bran contains 1.3 - 1.5 times as much nutritive material as fermented bran on a dry weight basis. In making these calculations, it is assumed that the portion of the wheat bran left unconverted by the fungus was also unavailable to the shrimp (see above, this section) and that the portion of the bran which was accessible to the fungus was converted at 40-50% efficiency. If these speculative calculations are fair approximations, then the fungal product may have actually given higher feed-conversion efficiency than the unfermented product, based on weight of nutritive matter added. Experiments involving feeding of purely fungal materials (no residual unfermented matter) or with more efficiently converted substrates (less residual unfermented matter) would be necessary to verify this conjecture.

In order to partially correct the differences in quantity of nutritive matter among fermented and unfermented bran feed in F74C and F74D, we applied feeding treatments of 50% fungal material and 50% unfermented bran. We did so for the further reason that plant starch has been found to be a valuable component of artificial penaeid diets (Andrews and Sick, 1973), and wheat bran starch and other nutritive components, as discussed above, obviously serve well in this capacity. The result of this mixing of fermented and unfermented feeds in F74C was to improve the maximal yield of shrimp, using a Pestalotia product, from 0.7 that of unfermented bran to slightly better than equivalency (Table 12). If half the smaller end of the correction-factor range (1.3) discussed in the above paragraph is applied to this yield, feed efficiency becomes nearly equal to what had been achieved with the Purina Experimental Marine Ration 20 in F74A. Feed efficiency was slightly lower in F74C than in F74A for unfermented bran, indicating that the Pestalotia + unfermented bran feeding treatment performed on a par with the Purina product, on the basis of nutritive matter fed (assuming that the ratio of shrimp yield from unfermented bran to shrimp yield from the Purina product would have been the same in F74C as it was in F74A).

The results of F74D demonstrate the marked effect which temperature (and salinity?) can have upon shrimp growth (Zein-Eldin and Griffith, 1969): feed efficiency for wheat bran rose to twice its previous low value, and mortalities were, in general, higher than in F74A and F74C. Yield values for wheat bran and the Purina product were much lower than in previous experiments, and this was not entirely due to the lower stocking density, for average final fresh weights were also distinctly lower. The mixtures of cold-stored Myrothecium product and dried Trichoderma product both performed less well than wheat bran in terms of shrimp yield, though the Myrothecium + unfermented bran product produced a yield which was only 4% less than the yield from unfermented bran. In addition to the ambient temperature problems, refrigeration problems occurred with the Myrothecium product in this experiment and probably further obscured real differences between this and other feeding treatments. Under the conditions of this experiment, particle size in the range tested (20 - 60 mesh) had no evident effect on shrimp yield from dried Trichoderma material.

The shrimp yields of our F74 series of feeding tests are low and the feed efficiencies high when compared with some of the results of other workers who have investigated growth of penaeids on artificial diets (Table 17). They are, however, comparable to the findings of Caillouet et al. (1975b) developed in the same concrete tanks as used in the present study. That they are a bit lower than Caillouet's findings is probably due to the fact that Caillouet used inorganic fertilizer in combination with his wheat bran feeding treatments, and we did not do so. There are two basic reasons for the low yields: (1) among the penaeid shrimp, Penaeus duorarum, used in F74A, B, and D, is one of the least attractive species in terms of growth potential under the conditions imposed by a mariculture facility (Broom, 1973; Parker and Holcomb, 1974; compare with Forster and Beard, 1974); (2) the facilities and methodology used in outgrowth of shrimp at Turkey Point have consistently brought about low yields when compared with other experimental facilities (see Krantz and Norris, 1976). Our results for these reasons and other experimental difficulties and faults discussed above, do not state nequivocally that fungal feeds can or should be used in mariculture of shrimp. ther, they suggest strongly that potential for development of a successful fungal ermented feed exists, especially in view of the

Table 17. Growth of penaeid shrimp as reported by several investigators. Only results of feeding artificial preparations are included. Present results are listed for experiments F 74A-D.

Reference	Species[a]	LGP[b]	In[c]	GMD[d] LO	GMD[d] HI (IW-FW)	F E[e] HI	F E[e] LO
Broom, 1969	*P. setiferus*	49-80	0.1 hect. ponds	0.01	0.97 (2.70-19.78)	4.9	3.0
	"	31-48	20 m^2 ponds	0.21	0.41 (1.46-2.42)	14.8	5.4
	P. aztecus	60-100	0.1 hect. ponds	0.11	0.70 (0.54-11.97)	9.7	1.7
	"	60	20 m^2 ponds	0.09	0.13 (1.10-4.42)	6.7	4.7
Neal & Latapie, 1973	*P. setiferus*	80-83	0.1 hect. ponds	0.27	1.09 (0.83-16.2)	3.2	1.1
	P. aztecus	62-100	"	0.08	0.47 (0.82-14.2)	12.6	1.6
Balazs *et al.*, 1973	*P. aztecus*	25	0.9 m^2 tanks	--	4.03 (0.58-1.20)	NR[f,g]	NR[g]
	P. japonicus	25	"	1.46	3.26 (1.50-6.44)	NR[g]	NR[g]
Shigeno *et al.*, 1972	*P. japonicus*	25	1.7 m^2 tanks	5.30	8.68 (0.94-2.83)	1.3[h]	1.0[h]
	"	50	"	4.16	7.02 (1.73-8.11)	2.0[h]	1.0[h]
Deshimaru & Shigeno, 1972	*P. japonicus*	60	1.7 m^2 tanks	12.71	15.13 (5.31-15.71)	1.6[h]	1.3[h]
Deshimaru & Kuroki, 1974a, b	*P. japonicus*	30	0.3 m^2 tanks	1.92	5.23 (0.87-2.49)	6.2	1.7
Andrews *et al.*, 1972	*P. setiferus*	56-70	0.8 m^2 tanks	0.07	0.28 (4-5.3)	NR	NR
Subrahmanyam & Oppenheimer, 1969	*P. a.* & *P. s.*	42	1 m^2 tanks	0.24	0.59 (0.61-1.28)	>100	5.5
Hysmith *et al.*, 1973	*P. a.*, postlarval	30	0.2 m^2 tanks	0.14	3.15 (0.06-0.29)	6.9[g]	1.6[g]
	P. a., juvenile	32	0.2 m^2 tanks	4.41	16.16 (3.04-8.33)	21.7[g]	1.4[g]
Parker & Holcomb, 1974	*P. s.*, juv., Table 2	85-100	0.2 hect. ponds	0.27	1.08 (0.29-10.81)	3.8	1.6

Table 17. (continued)

Reference	Species[a]	LGP[b]	In[c]	GMD[d] LO	GMD[d] HI (IW-FW)	F E[e] HI	F E[e] LO
Caillouet *et al.*, 1973	*P. duorarum*	51-64	2 m^2 tanks	0.07	0.40 (1.3-6.6)	48.1	7.8
	"	98	.2-.3 hect. ponds	0.18	0.32 (0.01-NR)	18.5	10.4
Caillouet *et al.*, 1975b	*P. duorarum* Exp. I	63	2 m^2 tanks	0.05	0.49 (0.01-NR)	17.1	2.2
	" Exp. II	63	"	0.10	0.31 (0.01-1.3)	17.9	5.6
F 74A, B, D, Purina "20"	*P. duorarum*	58-65	2 m^2 tanks	0.10	0.35 (0.01-1.35)	13	7
F 74A, B, D, wheat bran	"	"	"	0.08	0.27 (0.01-1.19)	16	9
F 74A, B, D, fungal feeds	"	"	"	0.04	0.20 (0.005-1.02)	50	13
F 74C, wheat bran	*P. setiferus*	60	"	0.15	0.15 (3.27-5.12)	8	8
F 74C, fungal feeds	"	"	"	0.04	0.11 (3.27-4.53)	33	12
F 74C, fungal and bran	"	"	"	0.11	0.15 (3.27-5.34)	12	8

a Of the genus *Penaeus*.

b Length of growth period in days.

c Area of the bottom of containers in which growth took place.

d Production of shrimp, in grams/m^2/day. Range (LO-HI) is not necessarily the full range observed; rather, it is that calculated from tables of results given in the references cited. (IW-FW)= the average initial and final fresh weights observed for the high production value.

e F E = feed efficiency=total dry weight of feed/final fresh weight of shrimp. Range determined as for GMD.

f NR= not reported or determinable from the reference cited.

g Excess food removed daily.

h As footnote g, but only consumed food used in calculations, and weight of dead animals included.

fact that our findings are the result of a very small-scale research effort.

14. <u>Culture screening on delignified bagasse and straw</u>

In our use of an agar plate method of screening, we attempted to circumvent the expense of instrument analysis and abbreviate the length of time necessary to determine ability of strains of fungi to convert test substrates into mycelial biomass. We did not measure the width of the peripheral growth zone which Trinci (1971) has found to be the factor by which specific growth rate in submerged culture can be related to colony radial growth rate on solid media. Had we done so, we would have arrived at a more accurate estimate of rate of increase in mass of tested fungi on each substrate than with only measurement of colony radial growth rate. Rather, we took into account hyphal diameter and hyphal density and used these to arrive at an indicator of fungal biomass produced. As Morrison and Righelato (1974) have found, hyphal density and hyphal diameter are important determinants of the width of the peripheral growth zone--it may be that refinement of methodology could yield an estimate of specific growth rate using measures of these two characters rather than attempting the more exacting techniques of Trinci (1971).

There are objections which can be raised to the use of our methodology in this culture screen. Eslyn (1969), Nilsson (1974), Sharp and Eggins (1970) demonstrated that assay of fungal cellulolysis may yield different results when agar-plate methods and other more direct methods are used. These studies, however, did not involve attempts to measure biomass. Another important fault lies in the fact that differences among test fungi in depth of penetration into the agar were not measured (a few marked differences were noted), and surface density and hyphal diameter undoubtedly do not reflect submerged characters to the same extent among strains of fungi. Whether or not our method achieved approximation of conversion ability would be best determined by conducting correlation analysis of fermentation results (protein production, chitin production, available energy production) with our (or alternative) computed indicators.

15. Future directions

When we undertook this project, it was the feeling of some of those already involved in the artificial detrital feed research, and some of those who were to sponsor our research, that the problem to be treated was a rather simple one: just grow up some fungi on an inexpensive substrate, submit it to the young shrimp, and raise them to marketable size. After all, they were eating the same stuff in nature, weren't they, and growing just fine there? And fungi have been growing on lignocellulosic materials in nature for eons. All that would be necessary would be to bring the process into the laboratory and accelerate it.

Perhaps one of our most significant findings is that the problem of developing a successful process for producing and utilizing natural food analogues is not as simple as it may seem on the surface. The series of steps in a functioning process is diagrammed in Fig. 12. As Calam (1969a) points out, research into particular fermentation processes has demonstrated "how stubborn and complex a fermentation project can be," and moving across Fig. 12 to introduction of the feed, the recent studies of L. V. Sick and his colleagues (Sick and Baptist, 1973; Sick et al., 1973) show that even inducing satisfactory ingestion rates can be a very complex problem. Regnault et al. (1975) should be consulted regarding problems of form of feed materials.

In order to successfully develop these steps in Fig. 12 so that they function as a working series, several factors or variables must be considered for each step. In many cases, the variables considered under a given step will have significant interacting effects on the functioning of that step, and to further complicate matters, variables which must be considered under one step may have distinct effects on the functioning of another step. We have given examples of this in Sections 8 and 12. As a further example, suppose we were to identify a fungus capable of converting a treated lignocellulosic substrate under a given set of fermentation conditions such that the fermented product contained a theoretically optimal amino acid-reserve-carbohydrate-lipid-chitin balance, examining as many variables under each of the steps in Fig. 12 as possible. After having done all of this exhaustive work, our product might well turn out to be a dismal failure, returning us to our starting position, because of mycelial unpalatability or even toxicity to our target aquacultured organism; Nikolei (1961) found that unpalatability and/or toxicity appeared to be the causes of failures of his gall-fly larvae to grow well on some of his fungal feeds, and that this could be caused by strain of fungus and/or fungal culturing conditions!

With a little imagination and pessimism, one can conjure up several other discouraging scenarios. On the other hand, if the problem is dealt with intelligently, it should be amenable to solution. We would propose that success not be attempted in leaps and bounds (as it was in the present study--success under these conditions could only be the result of serendipity); rather, careful, thorough analysis of some of the most basic questions should be the primary effort. The three major goals of early work would be fungal strain and substrate selection, determination of optimal delignification method, and determination of optimal type of fermentation.

Fungal strain(s) which would serve well in the process must be capable of both efficient conversion of the substrate used and satisfactory support of shrimp growth. Therefore, we feel that the problem of strain selection should be dealt with by conducting a dual screen of as many strains as possible, examining concomitantly the degree of ability of the fungi for substrate conversion and acceptability and nutritive capacity of the mycelia for the shrimp. Those which ranked high in both categories would then be used in development of the fermentation and feed preparation processes. The thermophilic fungi make prime initial candidates for this survey (Barnes et al., 1972; Cooney and Emerson, 1964; Romanelli et al., 1975). Genetic engineering (Esser, 1974) and alteration of fermentation conditions could then be used as tools in improvement of the fermentation product in terms of conversion efficiency and nutritive capacity. A listing of cultures of potentially biodeteriogenic fungi available at three major culture collections is given by Denizel et al. (1974).

Substrate selection would be based on the criteria of low acquisition cost and relative facility of conversion into fungal biomass. Substrates of low acquisition cost would be those agricultural by-products of high content of lignified cellulose and thereby little value as ruminant feed. They would have to be readily available in or near areas in which mariculture of shrimp has likelihood of commercial success in order that collection and transportation costs be minimal. They would have to be amenable to simple, low-cost delignification processes such as some of those which have been developed by the U. S. Environmental Protection Agency (Rogers and Spino, 1973) and other investigators (e.g. Bender et al., 1970; Han et al., 1975; Hartley et al., 1974; Heany et al., 1973; the work of Bavor (1974) may also be of interest here) in order to permit development of a fermentation or rumination process with high conversion rates.

Sugar cane bagasse is an example of an agricultural by-product which may meet the substrate-selection criteria. It is grown in large quantities in tropical climes where temperature conditions and land and labor costs are most favorable to penaeid shrimp mariculture operations. It is attractive as a substrate because it is available at centralized locations in the sugar processing plants (Callihan and Dunlap, 1969), and has limited value as feed, fuel, paper, or construction material (Srinivasan and Han, 1969). Simple delignification processes have been used on bagasse with success in increasing markedly its susceptibility to both bacterial and fungal degradation (from 15% to 70-80%) (Callihan and Dunlap, 1971; Dunlap, 1969; Garcia-Martinez et al., 1974). (It must be noted here that Cruz et al. (1967) claimed to have produced fungal-fermented bagasse of 28% crude protein without delignification and without adding a nitrogen source to the fermentation medium; their methods are cursorily reported,

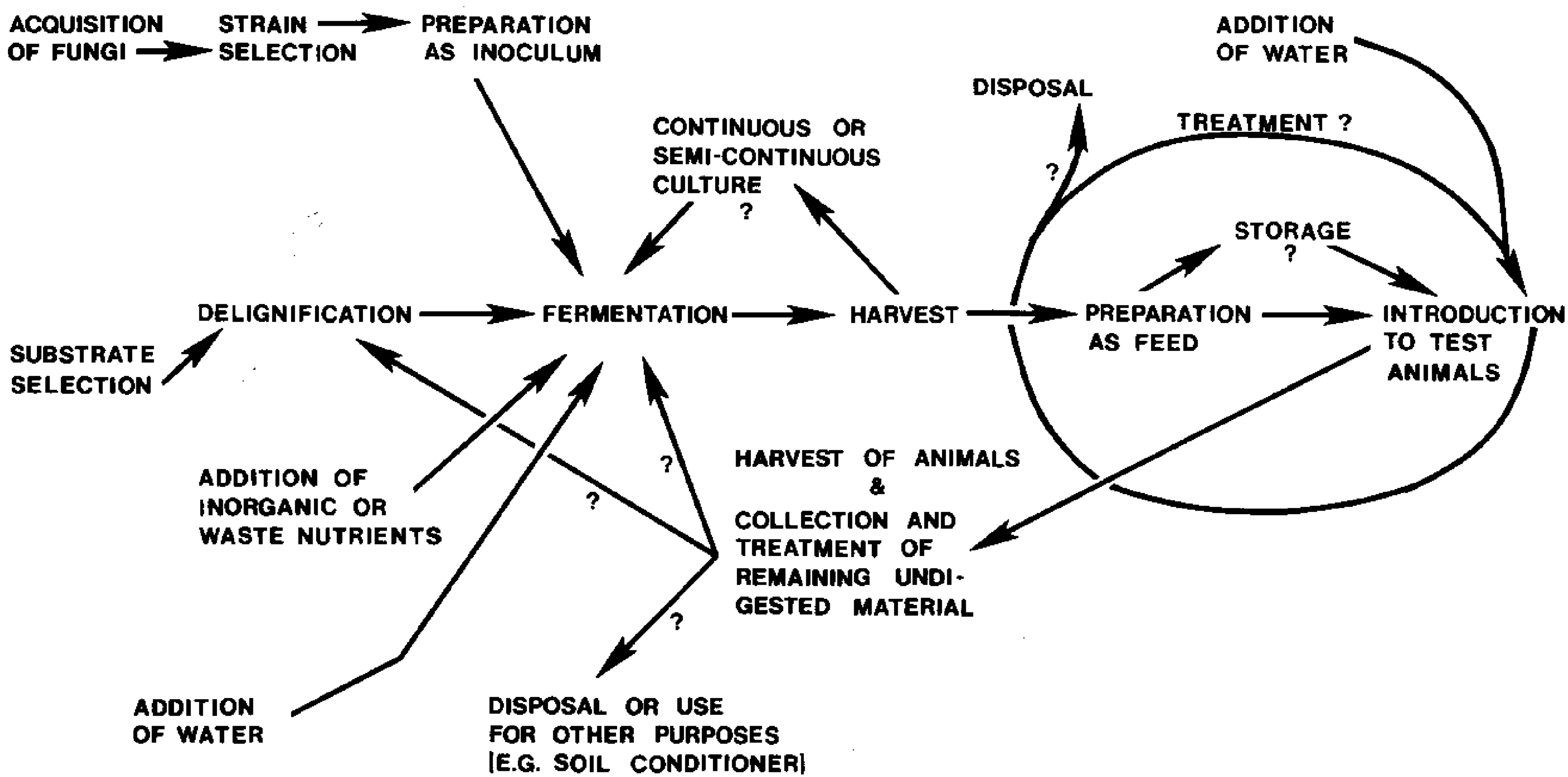

Figure 12. The basic series of steps in a hypothetical functioning artificial detrital aquacultural process.

and their results are of questionable accuracy.) See Mandels et al. (1974) for a review of the susceptibility of cellulosic materials to fungal lysis.

The choice of type of fermentation process which is to be used should be dictated by potential costs. A comparison of two basic types of fermentation processes, submerged-culture and solid-state, indicate that the latter is best suited for artificial detrital feed production. By "solid-state" fermentations we simply mean those which require only moistening, rather than submerging of the substrates to be fermented. Hesseltine (1972) has recently discussed the use of solid-state fermentations, and laments the fact that in the West, deep-tank, or submerged-culture, fermentation methods have been developed and used to nearly the exclusion of solid-state methods (a strong indication of this is the fact that solid-state apparatus is not dealt with in the reviews of fermentation design given by Blakebrough (1969), Solomons (1969), or Steel and Miller (1970)). Hesseltine lists advantages of solid-state fermentations; the most important and basic of these is the most obvious--solid-state fermentations require only small volumes of water. This removes the necessity for large vessels, and simplifies aeration methodology. It eliminates the need for supplies of large volumes of water and the problem of its disposal (if inorganic nutrients are supplied in the proper concentration, washing of the product of the fermentation should not be necessary). It provides conditions which in themselves inhibit bacterial contamination (the fungi are favored by low moisture levels relative to the bacteria (Hesseltine, 1972; Gray and Williams, 1971)). Harvesting of the product is clearly a very simple matter relative to the harvesting problems of submerged-culture fermentation; the finished product could conceivably be fed directly to the animals to be cultured.

Each of these advantages is a partial reason for the adaptability of solid-state fermentation to low-level technological operation (this aspect is discussed by N. J. Poole in a privately communicated manuscript which is now in preparation for publication—Poole and Smith, 1975; see also Imrie, 1975). As Brook et al. (1969) point out, solid-state fermentations are particularly well adapted to be carried out with simple apparatus at low cost, since this method of fermentation has been traditionally used in the production of fermented foods such as tempeh for over a thousand years (Gray, 1970; Hesseltine, 1965). What it amounts to is the production of a "vegetable cheese" (term from Brook et al.) with emphasis on production of mycelium. An example of a pilot-plant process similar to that which we envision for use in production of artificial detrital feeds is described for tempeh production by Steinkraus et al. (1965) and for production of "mold bran" by Underkofler et al. (1947). The sim licity of these processes permit them to be operated and managed by persons ... of mini al technical skill and training; this advantage has been recently recognized by Tcho anoglous et al. (1976), who discuss the likelihood that successful mariculture of crusta ea will probably involve use of highly labor-intensive plans. That the lignocellu sic substrates which we have recommended could be adapted to fermenta ons of this kind is evident from the success achieved by Hesseltine et al. (1968) in e production of secondary metabolites on forage materials (including oat straw) us g solid-state fungal fermentation, and from the preliminary results of Barnes et l. (1972) with solid-state fungal fermentation of paper and of N. J. Poole and his c leagues (personal communication) with solid-state fungal fermentation of straw (se Bery, 1972).

It w uld seem that the potential for development of valuable information from research to artificial detrital feeds is strongly indicated. If enough support for projects nilar to ours is forthcoming, and if thorough analysis of the set of basic problems volved in developing a successful process of production and utilization of these fe s is carried out (rather than hasty attempts at short-circuiting this fundamer l research), then we feel that it is likely that effective prototypal artificial etrital feeds will be developed in the near future.

16. Range of applicability

As a final note, it should be pointed out that artificial detrital feeds of the kind discussed above are not limited in their potential applicability to the husbandry of shrimp. Several other detritivorous or omnivorous animals are aquacultured and commercially valuable (e.g. carp, mullet, tilapia--see Bardach et al., 1972). These presumably (perhaps especially the mullets) have a digestive physiology geared to the types of feeds (microbial-detrital complexes) which would be produced by the methods which we propose. Feeds of this type might help change the fact that the "highest single cost factor" in raising low-cost food fishes such as these to marketable size is that of feed (Dassow and Steinberg, 1973). Other aquaculturable animals might also be able to grow satisfactorily on cheaply produced fungal protein if it were a component of their diets. W. D. Gray (personal communication) relates that tropical aquarium fish have been maintained for several months on a diet of fungal mycelium. Tiemeier and Deyoe (1973) propose, on the basis of their findings, that inexpensive vegetable protein sources be used in replacement of traditional animal sources in rearing of channel catfish. Sea lamprey larvae have been successfully raised using yeast as the only food source (Hanson et al., 1974). Even the apparently lucrative business of raising marine turtles (Anon., 1974) might benefit from application of this type of feed, since fungal feeds have been suggested for use with plant-eating livestock (Griffin et al., 1974) and growth of pigs on fungal protein has been shown to be equal to that produced by soybean meal and fishmeal (Duthie, 1975).

REFERENCES CITED

Agnihotri, V. P. 1964. Studies on aspergilli. Effect of pH, temperature, and carbon and nitrogen interaction. Mycopath. Mycol. Appl. 24: 305-314.

Anderson, C., J. Longton, C. Maddix, G. W. Scammell, and G. L. Solomons. 1975. The growth of microfungi on carbohydrates. In Third Int. Conf. on S. C. P. MIT Press, Cambridge, Mass. (in press).

Anderson, L. G. and D. C. Tabb. 1971. Some economic aspects of pink shrimp farming in Florida. Proc. Gulf Carib. Fish. Inst. 23: 113-124.

Andrews, J. W. and L. V. Sick. 1973. Studies on the nutritional requirements of penaeid shrimp. In Avault, J. W., Jr., E. Boudreaux, and E. Jaspers. (eds). Proc. Ann. Workshop World Mariculture Soc. 3: 403-414. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La.

Andrews, J. W., L. V. Sick, and G. J. Baptist. 1972. The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. Aquaculture 1: 341-347.

Anonymous. 1971. Fungi and unicellular algae as foods. Commonwealth Bur. Anim. Nutr. Annotated Bibliogr. No. 6. Aberdeen.

1974. Turtle livestock culture: a new food technology. Food Eng. 46(7): 58-59.

Balazs, G. H., E. Ross, and C. C. Brooks. 1973. Preliminary studies on the preparation and feeding of crustacean diets. Aquaculture 2(4): 369-377.

Balazs, G. H., E. Ross, C. C. Brooks, and ... Fujimura. 197... and level on growth of the captive freshwater prawn, Macr... In Avault, J. W., Jr. (ed). Ann. Workshop World Maricultur... State Univ. Div. Cont. Ed., Baton Rouge, La. (in press).

Bardach, J. E., J. H. Ryther and W. O. McLarney. 1972. Aquacu... and husbandry of freshwater and marine organisms. Wiley-... York. 868 pp.

Barlocher, F. and B. Kendrick. 1973. Fungi in the diet of Gamm... (Amphipoda). Oikos 24(2): 295-300.

1974. Dynamics of the fungal population on leaves in a s... 62(3): 761-791.

1975. Assimilation efficiency of Gammarus pseudolimnae... feeding on fungal mycelium or autumn-shed leaves... 59.

Barnes, T. G., H. O. W. Eggins, and E. L. Smith. 1972. Prelimina... development of a process for the microbial upgrading of w... Biodetn. Bull. 8(3): 112-116.

Basu, S. N. and S. N. Ghose. 1960. The production of cellulase by fungi on mixed cellulosic substrates. Can. J. Microbiol. 6: 265-282.

Bauer-Staeb, G. and F. Bouvard. 1973. An in vitro model for the determination of amino acid availability and digestion kinetics of protein-containing foods and its application to yeast. Lebensm. Wiss. Technol. 6(6): 219-223.

Bavor, H. J., Jr. 1974. Accelerated biodegradation of cellulosic substrates. Ph.D. dissertation. Louisiana State University, Baton Rouge, La.

Bednarski, W., J. Jakubowski, S. Poznanski, and A. Surazynski. 1970. Utilisation de la biosynthèse effectuée par des bactéries et des moisissures pour l'enrichissement des fourrages en protéines et vitamines à partir d'hydrates de carbonne. Lait 50: 285-303.

1971. Biosynthèse des protéines par des bactéries et des moisissures cultivées sur milieu de lactosérum. Lait 51: 638-652.

Bender, F., D. P. Heany, and A. Bowden. 1970. Potential of steamed wood as a feed for ruminants. Forest Prod. J. 20: 36-41.

Bery, M. K. 1972. Microbial upgrading of cellulosic materials. Ph.D. dissertation. University of Aston in Birmingham, England.

Blakebrough, N. 1969. Design of laboratory fermenters. In Norris, J. R. and D. W. Ribbons. (eds). Methods in microbiology. Vol. I: 473-504. Academic Press, New York.

Blumenthal, H. J. and S. Roseman. 1957. Quantitative estimation of chitin in fungi. J. Bacteriol. 74: 222-224.

Borzani, W., E. W. Leser, L. M. S. Venosa, and V. M. N. C. Martins. 1972. Sieving method for the determination of mold concentration in heterogeneous culture media. Biotechnol. Bioeng. 14(2): 273-277.

8. Microbiological breakdown of cellulose in the presence of arbon sources. J. Sci. Fd. Agric. 19: 133-135.

The use of yeast in human foods. In Mateles, R. I. and S. R. (eds). Single-cell protein: 90-121. MIT Press, Cambridge, Mass.

Stanton, and A. Wallbridge. 1969. Fermentation methods for ment of cassava. Biotechnol. Bioeng. 11(6): 1271-1284.

Pond culture of shrimp on Grand Terre Island, Louisiana, 1962- d Carib. Fish. Inst. Proc. 21: 137-151.

Bravery, A. F. alternativ

Bressani, R. 19 Tannenba

Brook, E. J., W. protein er

Broom, J. G. 19 1968. Gul

E. Boudreaux and E. Jaspers. (eds). Proc. An Soc. 3: 193-204. Louisiana State Univ. Div. La.

Brown, B. S., J. Mill, and J. M. Hulse. 1974. Chemical and biological degradation of waste plastics. Nature 250 (5462): 161-163.

Burnett, J. H. 1968. Fundamentals of mycology. Edward Arnold Ltd., London. 546 pp.

Caillouet, C. W., J. M. Heinen and D. C. Tabb. 1973. Pink shrimp feeding experiments with wheat bran. In Avault, J. W., Jr. E. Boudreaux and E. Jaspers (eds). Proc. Ann. Workshop World Mariculture Soc. 3: 415-460. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La.

Caillouet, C. W., J. P. Norris, E. J. Heald, and D. C. Tabb. 1975a. Growth and yield of pink shrimp (P. duorarum Burkenroad) in a feeding experiment in ponds. In Avault, J. W., Jr. (ed). Proc. Ann. Workshop World Mariculture Soc. 5. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La. (in press).

1975b. Growth and yield of pink shrimp (Penaeus duorarum Burkenroad) in feeding experiments in concrete tanks. Progr. Fish Cult. (in press).

Calam, C. T. 1969a. Culture of microorganisms in liquid medium. In Norris, J. R. and D. W. Ribbons. (eds). Methods in microbiology. Vol. I: 255-326. Academic Press, New York.

1969b. The evaluation of mycelial growth. In Norris, J. R. and D. W. Ribbons. (eds). Methods in microbiology. Vol. I: 567-591. Academic Press, New York.

Callihan, C. D. and C. E. Dunlap. 1969. The economics of microbial proteins produced from cellulose waste. Compost Sci. 10(1-2): 6-12.

1971. Construction of a chemical-microbial pilot plant for production of single-cell protein from cellulosic wastes. U. S. E. P. A. Report SW-24c. U. S. Environmental Protection Agency, Washington, D. C. 126 pp.

Chah, C. C., C. W. Carlson, G. Seminiuk, I. S. Palmer, and C. W. Hesseltine. 1973. The beneficial effects of some Aspergillus cultures for chick growth. Poult. Sci. 52(5): 2009.

Chahal, D. S. and S. P. S. Cheema. 1971. Production of fungal protein from rice flour. Plant. Sci. 3: 86-92.

Chahal, D. S. and W. D. Gray. 1968. Growth of selected cellulolytic fungi on wood pulp. In Walters, A. H. and J. S. Elphick. (eds). Biodeterioration of materials-microbiological and allied aspects: 584-593. Elsevier Publ. Co., London.

1970. Growth of cellulolytic fungi on wood pulp. II. Effect of different sugars and different levels of nitrogen on production of f
ngal protein.
Indian Phytopathol. 23: 74-79.

production
of organic

Chahal, D. S., G. D. Munshi, and S. P. S. Cheema. 1969. Fungal protei by Rhizoctonia solani when grown on cellulose as the sole source carbon. Proc. Nat. Acad. Sci. India 39B: 287-291.

Chahal, D. S., B. S. Sidhu, and A. H. Chora. 1972. Essential amino acid content of Penicillium crustosum Thom grown on rice flour as a carbon source. I abdev. J. Sci. Technol. B. Life Sci. 10(1): 41-46.

Chebotarev, L. N. and A. A. Zemlyanukhin. 1973. Assimilation of mineral nitrogen and protein biosynthesis in fungi under the action of visible light. Microbiology (USSR) 42(6): 884-886.

Chiao, J. S. and W. H. Peterson. 1953. Yeasts, methionine and cystine contents. Agricultural and Food Chemistry 1: 1005-1008.

Child, J. J., C. Knapp, and D. E. Eveleigh. 1973. Improved pH control of fungal culture media. Mycologia 65(5): 1078-1086.

Christias, C., C. Couvaraki, S. G. Georgopoulos, B. Macris, and V. Vomvoyanni. 1975. Protein content and amino acid composition of certain fungi evaluated for microbial protein production. Appl. Microbiol. 29(2): 250-254.

Church, B. D., H. A. Nash, and W. Brosz. 1972. Use of fungi imperfecti in treating food processing wastes. In Murray, E. D. (ed). Developments in Industrial Microbiology 13: 30-46. AIBS, Washington, D. C.

Cochrane, V. W. 1958. Physiology of fungi. John Wiley and Sons, New York. 524 pp.

Codner, R. C. 1969. Solid and solidified growth media in microbiology. In Norris, J. R. and D. W. Ribbons. (eds). Methods in Microbiology. Vol. I: 427-454. Academic Press, New York.

Coleman, W. E. 1972. Automatic amino acid analyses for determining the amount and quality of protein in fungal protein and in other protein sources. In Inglett, G. E. (ed). Symposium: seed proteins: 277-283. AVI Publishing Co., Inc., Westport, Conn.

Condrey, R. E., J. G. Gosselink, and H. J. Bennett. 1972. Comparison of the assimilation of different diets by Penaeus setiferus and P. aztecus. Fishery Bull. 70(4): 1281-1292.

Cook, H. L. and M. J. Lindner. 1970. Synopsis of biological data on brown shrimp, Penaeus aztecus aztecus Ives 1891. FAO Fish Rep. 4(57): 1471-1497.

Cooke, W. B. 1973. The usefulness of degradative fungi. Mycopath. Mycol. Appl. 51(2-3): 199-205.

Cooney, D. G. and R. Emerson. 1964. Thermophilic fungi. An account of their biology, activities, and classification. W. H. Freeman and Co. San Francisco. 188 pp.

Costello, T. J. and D. M. Allen. 1970. Synopsis of biological data on the pink shrimp Penaeus duorarum duorarum Burkenroad 1939. F.A.O. Fish. Rep. 4(57): 1499-1537.

Cowling, E. B. 1970. Nitrogen in forest trees and its role in wood deterioration. Acta Univ. Upsal. 164: 1-19.

Crawford, D. L., E. McCoy, J. M. Harkin, and P. Jones. 1973. Production of microbial protein from waste cellulose by Thermomonospora fusca, a thermophilic actinomycete. Biotechnol. Bioeng. 15: 833-843.

Cruz, R. A., R. E. Macaisa, T. J. Cruz, and C. C. Pusag. 1967. Biological treatment of bagasse enhances its value as a soil conditioner or feed. Sugar News 43: 15-22.

Dall, W. 1967. Food and feeding of some Australian penaeid shrimp. F.A.O. Fish. Rep. 2(57): 251-258.

Dassow, J. A. and M. A. Steinberg. 1973. The technological basis for development of aquaculture to produce low-cost food fish. Mar. Fish. Rev. 35(11): 6-13.

Denizel, T., B. Jarvis, A. H. S. Onions, A. C. Rhodes, R. A. Samson, E. G. Simmons, M. T. Smith and E. H. Huelk-Van Der Plas. 1974. A catalogue of potentially biodeteriogenic fungi held in the culture collections of the CBS (Centraalbureau voor Schimmelcultures), CMI (Commonwealth Mycological Institute) and QM (U.S. Army Natick Laboratories). Int. Biodetn. Bull. 10(1): 3-23.

Deshimaru, O. and K. Kuroki. 1974a. Studies on a purified diet for prawn-I. Basal composition of diet. Bull. Jap. Soc. Sci. Fish. 40(4): 413-421.

1974b. Studies on a purified diet for prawn-II. Optimum contents of cholesterol and glucosamine in the diet. Bull. Jap. Soc. Sci. Fish. 40(4): 421-425.

1974c. Studies on a purified diet for prawn-III. A feeding experiment with amino acid test diets. Bull. Jap. Soc. Sci. Fish. 40(11): 1127-1133.

1975. Studies on a purified diet for prawn-IV. Evaluation of protein, free amino acids and their mixture as nitrogen source. Bull. Jap. Soc. Sci. Fish. 41(1): 101-104.

Deshimaru, O. and K. Shigeno. 1972. Introduction to the artificial diet for prawn Penaeus japonicus. Aquaculture 1(1): 115-133.

Doctor, V. M. and L. Kerur. 1968. Penicillium mycelium waste as protein supplement in animals. Appl. Microbiol. 16: 1723-1726.

Dunlap, C. E. 1969. Proteins from waste cellulose by chemical microbial processing. Ph.D. dissertation. Louisiana State Univ., Baton Rouge, La. 268 pp.

Duthie, I. F. 1975. Animal feeding trials with a microfungal protein. In Third Int. Conf. on S.C.P. MIT Press, Cambridge, Mass. (in press).

El-Akher, M. A. A., A. M. Alian and N. M. El-Shimy. 1974. Production of high-protein
ey—Part I. Physical studies on cultivation feed additives from fermented w
. Technol. Lebensm. 3(3): 81-84. of yeast in whey. Chem. Microb

El-Kersh, T. A., M. A. Toama, and M. Abd El-Aziz. 1973. Cellulose production by Phoma glomerata and Rhizoctonia solani: I. Optimal conditions for the production of the enzyme. Chem. Microbiol. Technol. Lebensm. 2(4): 102-106.

Ernst, R. R. 1968. Sterilization by heat. In Lawrence, C. A. and S. S. Block (eds). Disinfection, sterilization, and preservation: 703-740. Lea and Febiger, Philadelphia.

Eslyn, W. E. 1969. A new method for appraising decay capabilities of microorganisms from wood chip piles. U. S. Forest Serv. Res. Pap. FPL 107: 1-8.

Ghandi, A. P., G. K. Barat, and N. B. Das. 1974. Studies on the production of fungal amylases. Mycopathol. Mycol. Appl. 52(3/4): 307-31[illegible]

Ghose, S. N. and K. W. King. 1963. The effects of physical and chemical properties of cellulosic fibers on anaerobic deterioration by pure culture. Textile Res. J. 33: 392-398.

Gould, R. F. (ed). 1969. Cellulases and their applications. Advances in Chemistry Series 95. American Chemical Society, Washington, D. C. 470 pp.

Grahan, D. C. W. 1971. The effects of cultural conditions on the production of protein and amino acids by certain fungi. Ph.D. dissertation. Cornell University. 94 pp.

Gray, T. R. G. and S. T. Williams. 1971. Soil microorganisms. Hafner Publ. Co., New York. 240 pp.

Gray, W. D. 1970. The use of fungi as food and in food processing. Vol. I. CRC Press, Cleveland, Ohio. 114 pp.

Gray, W. D. and M. Abou-El-Seoud. 1966a. Fungal protein for food and feeds. II. Whole sweet potato as a substrate. Econ. Bot. 20: 119-126.

1966b. Fungal protein for food and feeds. III. Manioc as a potential crude raw material for tropical areas. Econ. Bot. 20: 251-255.

1966c. Fungal protein for food and feeds. IV. Whole sugar beets or beet pulp as a substrate. Econ. Bot. 20: 372-376.

Gray, W. D. and M. D. Karve. 1967. Fungal protein for food and feeds. V. Rice as a source of carbohydrate for the production of fungal protein. Econ. Bot. 21: 110-114.

Gray, W. D., F. F. Och, and M. Abou-El-Seoud. 1964. Fungi imperfecti as potential sources of edible protein. Devel. Industr. Microbiol. 5: 384-389.

Gray, W. D., P. V. G. Pinto, and S. G. Pathak. 1963. Growth of fungi in seawater medium. Appl. Microbiol. 11(6): 501-505.

Griffin, H. L., J. H. Sloneker, and G. E. Inglett. 1974. Cellulose production by Trichoderma viride on feedlot waste. Appl. Microbiol. 27(6): 1061-1066.

Gross, G. B. 1973. Shrimp industry of Central America, Caribbean Sea, and northern South America. For. Fish. Leafl. (NMFS) 73-1: 1-47

Han, Y. W., J. W. Pence, and A. W. Anderson. 1975. Dioxane treatment to improve microbial digestibility of cellulosic fibers. Appl. Microbiol. 29(5): 708-709.

Han, Y. W. and V. R. Srinivasan. 1968. Isolation and characterization of a cellulose-utilizing bacterium. Appl. Microbiol. 16: 1140-1145.

Hanson, L. H., E. L. King, Jr., J. H. Howell, and A. J. Smith. 1974. A culture method for sea lamprey larvae. Prog. Fish Cult. 36(3): 122-128.

Hargrave, B. T. 1970. The utilization of benthic microflora by Hyalella azteca (Amphipoda). J. Anim.Ecol. 39: 427-437.

Hartley, R. D., E. C. Jones, N. J. King, and G. A. Smith. 1974. Modified wood waste and straw as potential com... ents of animal feeds. J. Sci. Fd. Agric. 25(4): 433-437.

...ister. 1973. Use of steamed aspen poplar in ... steers. Can. J. Anim. Sci. 53: 739-740.

...strange. 1971. Chemical analysis of microbial ... Ribbons (eds). Methods in microbiology. ...ress, New York.

... of fungi, food and fermentation. Mycologia

...ns. Biotechnol. Bioeng. 14(4): 517-532.

... L. Smith, G. M. Shannon, E. E. Vandegraft, ... laboratory studies on the formation of aflatoxin ... (2): 304-312.

...lly controlled ...nce. In ... Fermentation

... digestion.

... field of ...nitrogen

...f the effects ...vault J. ...orld Mariculture ...e, La.

... Res. Bd.

...rus. J.

... 66(950):

...l pp.

...gi to salin ty.

Heany, D. P., F. Bender, and E. ... a finishing ration for Holst...

Herbert, D., P. J. Phipps, and R. ... cells. In Norris, J. R. and D... Vol. 5B: 209-344. Academ...

Hesseltine, C. W. 1965. A millen... 57: 149-197.

1972. Solid state ferment...

Hesseltine, C. W., O. L. Shotwell ... and M. ... Goulden. ... in forages. Mycologi...

Hishinuma, K., Y. Iida, K. Kobayashi, and S. Ikeda. 1972. An automa... miniature fermenter for laboratory use--system design and expe... Terui, G. (ed). Fermentation technology today: 155-161. Societ... Technology, Osaka, Japan.

Hood, M. A. and S. P. Meyers. 1974. Microbial aspects of penaeid shr... Proc. Gulf Carib. Fish. Inst. 26: 81-91.

Hueck, H. J. and W. Hazeu. 1969. Experience with biological tests in... the biodeterioration of materials. 4. The influence of carbon a... sources in mildew test media. Int. Biodetn. Bull. 5(4): 153-161.

Hysmith, B. T., J. R. Booth, H. L. Cook, and W. L. Mies. 1973. A stu... of feeding synthetic diets to brown shrimp (Penaeus aztecus). I... W. Jr., E. Boudreaux, and E Jaspers. (eds). Proc. Ann. Worksho... Soc. 3: 365-389. Louisiana State Univ. Div. Cont. Ed., Baton R...

Idyll, C. P. 1973. Marine aquaculture: problems and prospects. J. F... Can. 30(12(2)): 2178-2183.

Imrie, F. K. E. 1973. The production of fungal protein from carob in ... Sci. Fd. Agric. 24(5): 639.

1975. Single-cell protein from agricultural wastes. New Scien... 458-460.

Jones, A. 1974. World protein resources. Halstead Press, New York.

Jones, E. B. G., A. Byrne, and D. J. Alderman. 1971. The response of ... Vie et Milieu Suppl. No. 22: 265-280.

Jones, E. B. G. and J. Irvine. 1971. The role of marine fungi in the biodeterioration of materials. Int. Biodeterioration Symposium 2: 422-431. App. Sci. Publ. Ltd., Barking, England.

Jones, R. R. 1974. Utilization of Louisiana estuarine sediments as a source of nutrition for the brown shrimp, Penaeus aztecus. Ph.D. dissertation. Louisiana State University, Baton Rouge, La.

Joshi, G. P. 1974. The biology of Stictothrips fimbriata Anan., a mycophagous Thysanoptera on different meals. Z. Angew. Entomol. 76(2): 146-149.

Kanazawa, A., M. Shimaya, M. Kawasaki, and M. Kashiwada. 1970. Nutritional requirements of prawn-I. Feeding on artificial diet. Bull. Jap. Soc. Sci. Fish. 36: 949-954.

Kanazawa, A., N. Tanaka, S. Teshim[illegible] and K. Kashiwada. 1971. Nutritional requirements of prawn-II. Requirement for sterols. Bull. Jap. Soc. Sci. Fish. 37: 211-215.

Kaushik, N. K. and H. B. N. Hynes. 1971. The fate of dead leaves that fall into streams. Arch. Hydrobiol. 68: 465-515.

Kihlberg, R. 1972. The microbe as a source of food. Ann. Rev. Microbiol. 26: 427-466.

Kilgen, R. and A. Harris. 1975. Over-wintering of white shrimp in estuarine impoundments in Louisiana. In Avault, J. W. Jr. (ed). Proc. Ann. Workshop World Mariculture Soc. 5. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La. (in press).

King, B. and H. O. W. Eggins. 1973. [illegible] mechanisms of microfungi which might produce an enhanced permeability in wood. Int. Biodetn. Bull. 9(1/2): 35-43.

Kitabayashi, K., H. Kurata, and S. Ishikaw[illegible]. 1971a. Studies on formula feed for Kuruma prawn. I. On the relationship among glucosamine, phosphorus, and calcium. Bull. Tokai Reg. Fish. Res. Lab. 65: 91-105.

Kitabayashi, K., K. Shudo, K. Nakamaru, [illegible] and S. Ishikawa. 1971b. Studies on the formula feed of Kuruma prawn. II. [illegible] the growth promoting effects of both [illegible]

LéJohn, H. B. 1971. Enzyme regulation, lysine pathways and cell wall structures as indicators of major lines of evolution in fungi. Nature 231: 164-168.

Levi, M. P. and E. B. Cowling. 1969. Role of nitrogen in wood deterioration. 7. Physiological adaptation of wood destroying and other fungi to substrates deficient in nitrogen. Phytopathol. 59(4): 460-468.

Lilly, V. G. 1965. Chemical constituents of the fungal cell. In Ainsworth, G. C. and A. S. Sussman. (eds). The fungi. An advanced treatise. Volume I. The fungal cell: 163-178. Academic Press, New York.

Ling, S. W. 1969. Methods of rearing and culturing Macrobrachium rosenbergii (De Man). FAO Fish. Rep. 3(57): 607-619.

Litchfield, J. H. 1968. The production of fungi. In Mateles, R. I. and S. R. Tannenbaum. (eds). Single-cell protein: 309-329. MIT Press, Cambridge, Mass.

Litchfield, J. H., R. C. Overbeck, and R. J. Davidson. 1963. Factors affecting the growth of morel mushroom mycelium in submerged culture. J. Agr. Food Chem. 11(2): 158-162.

Mandels, M., L. Hontz, and J. Nystrom. 1974. Enzymatic hydrolysis of waste cellulose. Biotechnol. Bioeng. 16(11): 1471-1493.

Marasas, W. F. O. and E. B. Smalley. 1972. Mycoflora, toxicity and nutritive value of mouldy maize. Onderstepoort J. Vet. Res. 39(1): 1-10.

Mateles, R. I. and S. R. Tannenbaum (eds). 1968. Single-cell protein. MIT Press, Cambridge, Mass. 480 pp.

Mateles, R. I. and E. Battat. 1974. Continuous culture used for media optimization. Appl. Microbiol. 28: 901-905.

Mathews, C. P. and A. Kowalczewski. 1969. The disappearance of leaf litter and its contribution to production in the River Thames. J. Ecol. 57: 543-552.

May, M. S. III. 1974. Probable agents for the formation of detritus from the halophyte, Spartina alterniflora. In Reimold, R. J. and W. H. Queen. (eds). Ecology of halophytes: 429-440, Academic Press, New York.

Merrill, W. and R. B. Cowling. 1966. Role of nitrogen in wood deterioration. Amount and distribution of nitrogen in fungi. Phytopathol. 56: 1083-1090.

Meyers, S. P. 1968. Degradative activities of filamentous marine fungi. Proc. Int. Biodeterioration Symp. 1: 594-609. Elsevier, England.

1971. Developments in the biology of filamentous marine fungi. In Jones, E. B. G. and S. K. Eltringham (eds). Marine borers, fungi, and fouling organisms of wood: 217-235. O.E.C.D., Paris.

Meyers, S. P., J. W. Avault, Jr., J. S. Rhee, and D. Butler. 1970. Development of rations for economically important aquatic and marine invertebrates. Coastal Studies Bull. 5: 157-172.

Meyers, S. P. and Z. P. Zein-Eldin. 1973. Binders and pellet stability in development of crustacean diets. In Avault, J. W., Jr., E. Boudreaux, and E. Jaspers (eds). Proc. Ann. Workshop World Mariculture Soc. 3: 351-364. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La.

Minami, Z., M. Kajita, and S. Hibino. 1972. Studies on the utilization of petro-yeast as diet of freshwater fish. I. The distribution of chitin decomposing bacteria in digestive tracts. Bull. Jap. Soc. Sci. Fish. 38(9): 1013-1020.

Mirocha, C. J. and C. M. Christensen. 1974. Fungus metabolites toxic to animals. In Baker, K. F. (ed). Annual review of phytopathology 12: 303-330. Annual Reviews, Inc., Palo Alto, Cal.

Mock, C. R. 1973. The culture of Penaeus japonicus in Japan. In Avault, J. W. Jr., E. Boudreaux, and E. Jaspers (eds). Proc. Ann. Workshop World Mariculture Soc. 3: 285-286. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La.

Mogren, H., G. Hedenskog, and A. Von Hofsten. 1973. The influence of heat processing and mechanical disintegration on yeast for single-cell protein. Physiol. Plant 29(1): 82-91.

Morrison, K. B. and R. C. Righelato. 1974. The relationship between hyphal branching, specific growth rate and colony radial growth rate in Penicillium chrysogenum. J. Gen. Microbiol. 81: 517-520.

Nagel-DeBoois, H. M. and E. Jansen. 1971. The growth of fungal mycelium in forest soil layers. Rev. Ecol. Biol. Sol. T8(4): 509-520.

Neal, R. A. 1973. Progress toward farming shrimp in the United States. Mar. Fish. Rev. 35(3-4): 67-70.

Neal, R. A. and [illegible]. 1973. Pond culture on Grand Terre Island, Louisiana, 1969-1971. In Avault, J. W. Jr., E. Boudreaux, and E. Jaspers (eds). Proc. Ann. Workshop World Mariculture Soc. 3: 227-240. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La.

Newell, S. Y. 1975. Mangrove fungi: the succession in the mycoflora of red mangrove (Rhizophora mangle L.) seedlings. In Jones, E. B. G. (ed). Recent advances in aquatic mycology: 51-91. Paul Elek Ltd., London.

Nicholas, D. J. D. 1965. Utilization of inorganic nitrogen compounds and amino acids by fungi. In Ainsworth, G. C. and A. S. Sussman. (eds). The fungi. An advanced treatise. I. The fungal cell: 349-376. Academic Press, New York.

Nikolei, E. 1961. Vergleichende Untersuchungen zur Fortpflanzung heterogoner Gallmücken unter experimentellen Bedingungen. Z. Morph. Ökol. Tiere 50: 281-329.

Nilsson, T. 1974. The degradation of cellulose and the production of cellulase, xylanase, mannanase, and amylase by wood-attacking microfungi. Stud. For. Suec. 114 : 1-61.

Odum, W. E. and E. J. Heald. 1972. Trophic analyses of an estuarine mangrove community. Bull. Mar. Sci. 22(3): 671-738.

Odum, W. E., J. C. Zieman, and E. J. Heald. 1973. Importance of vascular plant detritus to estuaries. In Chabreck, R. H. (ed). Proceedings of the coastal marsh and estuary management symposium: 91-114. Louisiana State Univ.

4. Growth and production of brown and P. setiferus) from experimental ponds in as. In Avault, J. W., Jr. and E. Boudreaux Mariculture Soc. 4: 215-234. Louisiana Rouge, La.

Parker, J. C. and H. W. Holcomb, Jr. white shrimp (Penaeus aztecus Braxoria and Orange Counties, (eds). Proc. Ann. Workshop Wo State Univ. Div. Cont. Ed., Bat

of Penicillium chrysogenum mycelium (2): 262-266.

Pathak, S. G. and R. Seshadri. 1965. as animal food. Appl. Microbio

nding, enzyme digestion, and the lignin : 247-256.

Pew, J. C. and P. Weyna. 1962. Fine cellulose bond in wood. Tappi 4

pocellulose in ruminant nutrition

Pigden, W. J. and D. P. Heaney. 1969.

Chem. Ser. 95: 245-261.

on issertation,

Pinto, P. V. C. 1963. The influence of substrate composition and environm the amino acid content of the mycelia of some fungi imperfecti. Ph.D Ohio State Univ. 78 pp.

Pirt, S. J. 1966. A theory of the mode of growth of fungi in the form of pe in submerged culture. Proc. Roy. Soc. B166: 369-373.

1967. A kinetic study of the mode of growth of surface colonies of bac and fungi. J. Gen. Microbiol. 47: 181-197.

script).

Poole, N. J. and A. L. Smith. 1975. The potential of low technology. (in ma

Porter, J. R. 1974a. Microbiology and the food and energy crises. ASM Ne 813-825.

1974b. Microbiology and the disposal of solid wastes. ASM News 826-833.

Prokop, A. and F. Stros. 1974. On the evaluation of productivity in hydroca fermentation. Folia Microbiol. 19(1): 38-42.

train

Reade, A. E., K. F. Gregory, G. L. Khor, and J. C. Alexander. 1974. Produc of single-cell protein from cassava by a thermophilic amylase-produci of Aspergillis. Abstracts Ann. Meeting A.S.M. 74.

Reade, A. E., R. H. Smith, and R. M. Palmer. 1972. The production of prote non-ruminant feeding by growing filamentous fungi on barley. Biocher 127(2): 32p.

Regnault, M., A. Campillo, and P. Lu[illegible]uet. 1975. Croissance des crevettes Crangon crangon and Palaemon serratus soumises à un régime artificiel: influence du mode de présentation et du mode de séchage de l'aliment. Cahiers Biol. Mar. 16(1): 1-20.

Rhodes, R. A., H. H. Hall, R. T. Anderson, G. E. N. Nelson, M. C. Shekleton, and R. W. Jackson. 1961. Lysine, methionine, and tryptophan content of microorganisms. III. Molds. Appl. Microbiol. 9(3): 181-184.

Richardson, L. R., S. Hayes, and R. H. Rigdon. 1967. The nutritive value of moldy grains and protein concentrates for growth of poults. Poult. Sci. 46: 168-176.

Roedel, P. M. 1973. Shrimp '73—a billion dollar business. Mar. Fish. Rev. 35(3/4): 1-2.

Rogers, C. J. and D. Spino. 1973. Microbial protein production from cellulosic wastes. In Inglett, G. E. (ed). Symposium: processing agricultural and municipal wastes: 161-171. AVI Publ. Co, Westport, Conn.

Romanelli, R. A., C. W. Houston, and S. M. Barnett. 1975. Studies on thermophilic cellulolytic fungi. Appl. Microbiol. 30(2): 276-281.

Rowley, B. I. and A. T. Bull. 1973. Chemostat for the cultivation of moulds. [illegible] Pract. 22(4): 286-289.

Sass, J. E. 1958. Botanical microtechnique. Iowa State Univ. Press, Ames, Io[illegible] 228 pp.

Schultz, D. M. and J. G. Quinn. 1973. Fatty acid composition of organic detri[illegible] from Spartina alterniflora. Estuarine and Coastal Mar. Sci. 1: 177-190.

Seal, K. J. and H. O. W. Eggins. 1972. The role of microorganisms in the biode[illegible] of farm animal wastes with particular reference to intensively produced [illegible] A review. Int. Biodetn. Bull. 8(3): 95-100.

Sekeri-Pataryas, K. E., K. A. Mitrakos, and M. K. Georgi. 1973. Yields of fun[illegible] [illegible] protein from caro[illegible]

Sguros, P. L., S. P. Meyers, and J. Simms. 1962. Role of marine fungi in the biochemis[illegible] of the oceans. I. Establishment of quantitative technique for cultivation, growth measurement, and production of inocula. Mycologia 54: 521-535.

Sguros, P. L., J. Rodrigues, and J. Simms. 1973. Role of marine fungi in the biochemis[illegible] of the oceans. V. Patterns of constitutive nutritional growth response. Mycolog[illegible] 65(1): 161-174.

Sguros, P. L. and J. Simms. 1963. Role of marine fungi in the biochemistry of the oceans. II. Effect of glucose, inorganic nitrogen, and tris (hydroxymethyl) aminomethane on growth and pH changes in synthetic media. Mycologia 55: 728-741.

Shacklady, C. A. 1972. Hydrocarbon fermentation. A new source of protein. World Anim. Rev. 3: 19-22.

Shang, Y. C. 1974. Economic feasibility of freshwater prawn farming in Hawaii. Hawaii Univ. Sea Grant Progr. Advis. Rep. UNIHI-SEA GRANT-AR-74 - 05. 64 pp.

Sharda, D. P., R. F. Wilson, L. E. Williams, and L. A. Swiger. 1971. Effect of feeding corn inoculated with Nigrospora oryzae and Cladosporium on the performance of growing swine and rats. J. Anim. Sci. 33(6): 1259-1267.

Sharp, R. F. and H. O. W. Eggins. 1970. The ecology of soft-rot fungi. I. Influence of pH. Int. Biodetn. Bull 6(2): 53-64.

Shewbart, K., W. Mier, and P. Ludwig. 1973. Nutritional requirements of brown shrimp, Penaeus aztecus. Texas A & M Univ. Sea Grant Publ. 205: 1-51.

Shigeno, K., K. Kumada, O. Deshima-u, T. Aramaki, K. Kuroki and K. Kitaue. 1972. Studies on the artificial diets of prawn—I. Relationship between the feed efficiency and crude protein in the diets. Bull. Jap. Soc. Sci. Fish. 38(2): 101-106.

abayashi. 1971. Studies on the formula
h-promoting effects of both squid
Fish. Res. Lab 65: 129-138.

of fungal protein from waste molasses.

of selected dietary lipids, carbohydrates,
dy composition of Penaeus duorarum.
ls). Proc. Ann. Workshop World
ate Univ. Div. Cont. Ed., Baton

cted physical and nutritional factors
arval penaeid shrimp. J. Elisha

tudy in formula food technology
hium larval and juvenile shrimps.
shop World Mariculture Soc. 5.
n Rouge, La. (in press).

effect of duration of feeding, amount
rate of ingestion of pelleted food
[illegible]

the behavioral sciences. McGraw-

f cellulose. With special reference
, New York. 531 pp.

Shudo, K., K. Nakamura, S. Ishikawa, and K.
feed for Kuruma prawn. IV. On the gr
liver oil and cholesterol. Bull. Tokai R

Shukla, J. P. and S. M. Dutta. 1967. Product
Indian J. Technol. 5: 27-28.

Sick, L. V. and J. W. Andrews. 1974. The eff
and proteins on the growth, survival an
In Avault, J. W., Jr., and E. Boudreaux
Mariculture Soc. 4: 263-276. Louisian
Rouge, La.

Sick, L. V. and G. Baptist. 1973. Effects of
on rates of pelleted diet ingestion by p
Mitchell Sci. Soc. 89(3): 161-165.

Sick, L. V. and J. Harris. 1975. A comparati
and application for penaeid anc Macrob
In Avault, J. W., Jr. (ed). Proc. Ann. W
Louisiana State Univ. Div. Cont. Ed., B

Sick, L. V., D. White, and G. Baptist. 1973.
of food, light intensity, and animal size
[illegible]

Siegel, S. 1956. Nonparametric statistics
Hill, New York. 312 pp.

Siu, R. G. H. 1951. Microbial decompositio
to cotton textiles. Reinhold Publ. C

Snedecor, G. W. and W. G. Cochran. 1967. Statistical methods. Sixth edition. Iowa State Univ. Press, Ames, Io. 593 pp.

Sokal, R. R. and F.J. Rohlf. 1969. Biometry. The principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco. 776 pp.

Solomons, G. L. 1969. Materials and methods in fermentation. Academic Press, New York. 331 pp.

1972. Improvements in the design and operation of the chemostat. J. Appl. Chem. Biotechnol. 22: 217-228.

1973. Fungal protein. J. Sci. Food Agric. 24(5): 637-638.

1975. Submerged culture production of mycelial biomass. In Smith, J. and D. Berry (eds). Industrial utilization of mycelial fungi: 249-263. Edward Arnold Ltd., London.

Solomons, G. L. and G. W. Scammell. 1974a. Improvements in or relating to microorganisms. British Patent Spec. 1346061.

1974b. Improvements in the production of edible protein containing substances. British Patent Spec. 1346062.

Spicer, A. 1971. Protein production by microfungi. Trop. Sci. 13(4): 239-263.

1973. Proteins from carbohydrates. Chem. in Britain 9(3): 100-103.

Srinivasan, V. R. and Y. W. Han. 1969. Utilization of bagasse. Adv. Chem. Ser. 95: 447-460.

Steel, R. and T. L. Miller. 1970. Fermentor design. In Perlman, D. (ed). Advances in applied microbiology 12: 153-188. Academic Press, New York.

Steinkraus, K. H., J. P. Van Buren, L. R. Hackler, and D. B. Hand. 1965. A pilot plant process for the production of dehydrated tempeh. Food Technol. 19(1): 63-68.

Subrahmanyam, C. B. and C. H. Oppenheimer. 1969. Food preference and growth of grooved penaeid shrimp. In Youngken, H. W. (ed). Food-drugs from the sea, proceedings: 65-75. Marine Technology Soc., Washington, D. C.

Tabb, D. C., W. T. Yang, C. P. Idyll, and E. S. Iversen. 1969. Research in marine aquaculture at the Institute of Marine Sciences, University of Miami. Trans. Amer. Fish. Soc. 98(4): 738-742.

Tabb, D. C., W. T. Yang, Y. Hirono, and J. Heinen. 1972. A manual for culture of pink shrimp, Penaeus duorarum, from eggs to postlarvae suitable for stocking. Univ. Miami Sea Grant Special Bull. 7: 1-59.

Tashpulatov, Z. and N. A. Teslinova. 1974. Biosynthesis of cellulolytic enzymes by Trichoderma lignorum depending on the conditions of culturing. Microbiology (USSR) 43(4): 516-519.

Taub, F. B. 1973. Nutrition workshop. Moderator's opening comments. In Avault, J. W., Jr., E. Boudreaux, and E. Jaspers (eds). Proc. Ann. Workshop World Mariculture Soc. 3: 349-350. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La.

Tchobanoglous, G., R. A. Shlesser, and T. M. Schuur. 1976. Energy and resource consumption in crustacean aquaculture. In Avault, J. W., Jr. (ed). Proc. Ann. Workshop World Mariculture Soc. 6. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La. (in press).

Thanh, N. C. and R. E. Simard. 1973. Biological treatment of domestic sewage by fungi. Mycopathol. Mycol. Appl. 51(2/3): [illegible].

Thatcher, F. S. 1954. Foods and feeds from fungi. Ann. Rev. Microbiol. 8: 449-472.

Thomke, S. 1972a. Deterioration processes in newly harvested grains. V. Experiment with barley deteriorated under controlled conditions and fed to broilers. Swedish J. Agric. Res. 2(1): 27-37.

1972b. Deterioration processes in newly harvested grains: VI. Experiments with barley deteriorated under controlled conditions and fed to mice and rats. Swed. J. Agric. Res. 2(1): 39-44.

Tiemeier, O. W. and C. W. Deyoe. 1973. Producing channel catfish [illegible]. Kansas Agric. Exp. Stn. 576: 1-24.

Tkachenko, V. V., S. S. Rylkin, A. N. Skidchenko, and V. E. Sterkin. 1972. Influence of culturing conditions on the fractional composition of microorganism proteins. Microbiology (USSR) 40(4): 568-571.

Torev, A. K. 1973. An industrial technology for production of higher fungi mycelium (in Bulgarian). Publ. House of the Bulgarian Acad. Sci., Sofia.

Toyama, N. and K. Ogawa. 1972. Utilization of cellulosic wastes by Trichoderma viride. In Terui, G. (ed). Fermentation technology today: 743-757. Society of Fermentation Technology, Osaka, Japan.

Trinci, A. P. J. 1969. A kinetic study of the growth of Aspergillus nidulans and other fungi. J. Gen. Microbiol. 57: 11-24.

1971. Influence of the width of the peripheral growth zone on the radial growth rate of fungal colonies on solid media. J. Gen. Microbiol. 67: 325-344.

Triska, F. J. 1970. Seasonal distribution of aquatic hyphomycetes in relation to the disappearance of leaf litter from a woodland stream. Ph.D. dissertation, Univ. of Pittsburgh. 189 pp.

Underkofler, L. A., G. M. Severson, K. J. Goering, and L. M. Christensen. 1947. Commercial production and use of mold bran. Cereal Chem. 24: 1-22.

Updegraff, D. M. 1971. Utilization of cellulose from waste paper by Myrothecium verrucaria. Biotechnol. Bioeng. 13: 77-97.

Van Soest, P. J. 1969. Composition, maturity, and the nutritive value for forages. Adv. Chem. Ser. 95: 262-278.

Venkataramiah, A., G. J. Lakshmi, and G. Gunter. 1975. Effect of protein level and vegetable matter on growth and food conversion efficiency of brown shrimp. Aquaculture 6: 115-125.

Verona, O., M. P. Nuti, and G. Anelli. 1973. Influence of some nitrogen sources on amino acid pool of 2 hyphomycetes. Experientia (Basel) 29(9): 1162-1164.

Von Hofsten, B. 1975. Cultivation of a thermotolerant basidiomycete on various carbohydrates. Int. Symp. on Food from Waste. Weybridge, U. K., 7-8 April, 1975 (in press).

Von Hofsten, B., and A. Von Hofsten. 1974. Ultrastructure of a thermotolerant basidiomycete possibly suitable for production of food protein. Appl. Microbiol. 27(6): 1142-1148.

Wang, H. L., J. B. Vespa, and C. W. Hesseltine. 1974. Acid protease production by fungi used in soybean food fermentation. Appl. Microbiol. 27(5): 906-911.

Ward, E. W. B. and N. Colotelo. 1960. Importance of inoculum standardization in nutritional experiments with fungi. Can. J. Microbiol. 6: 545-556.

Warnock, D. W. 1971. Assay of fungal mycelium in grains of barley, including the use of the fluorescent antibody technique for individual fungal species. J. Gen. Microbiol. 67: 197-205.

Webber, H. H. 1973. Risks to the aquaculture enterprise. Aquaculture 2(2): 157-172.

1975. Crustacean aquaculture in middle America. Mar. Fish. Rev. 37(1): 24-30.

Weiner, B. A. and R. A. Rhodes. 1974. Fermentation of feedlot waste filtrate by fungi and streptomycetes. Appl. Microbiol. 28(5): 845-850.

Weisman, E. 1970. Installation of fermentation pilot plant. Proc. Biochem. 5(2): 10-11, 18.

Willoughby, L. G. and K. Redhead. 1973. Observations on the utilization of soluble

...Mycol. Soc. ... nitrogen by aquatic fungi in nature. Tran

Wilson, R. K. and W. J. Pigden. 1964. Effect o the utilization of wheat straw and poplar Can. J. Anim. Sci. 44: 122-123.

a sodium hydroxide treatment on
ood by rumen microorganisms.

Worgan, J. T. 1968. Culture of the higher fung in industrial microbiology 8: 73-139. Chu

In Hockenhull, D. J. D. (ed). Progress
hill, London.

1973. Protein production by microorganisms from carbohydrate substrates. In Jones, J. G. W. (ed). The biological efficiency of protein production: 339-361. Cambridge Univ. Press, New York.

Yusef, H. M. and M. E. Allam. 1967. The carbon and nitrogen nutrition of certain fungi. Can. J. Microbiol. 13(8): 1097-1106.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J. 620 pp.

Zein-Eldin, Z. P. and G. W. Griffith. 1969. An appraisal of the effects of salinity and temperature on growth and survival of postlarval penaeids. FAO Fish. Rep. 3(57): 1015-1026.

Zein-Eldin, Z. P. and J. C. McGaffey. 1976. Protein quantity and quality in diets of penaeid shrimp. In Avault, J. W., Jr. (ed). Proc. Ann. Workshop World Mariculture Soc. 6. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La. (in press).

Zein-Eldin, Z. P. and S. P. Meyers. 1974. General consideration of problems in shrimp nutrition. In Avault, J. W., Jr. and E. Boudreaux (eds). Proc. Ann. Workshop World Mariculture Soc. 4: 299-317. Louisiana State Univ. Div. Cont. Ed., Baton Rouge, La.

Zeltin, R. P. 1970. Influence of the nutrient medium on the biosynthesis of cellulolytic enzymes of the thermotolerant fungus Aspergillus terreus. Microbiology (USSR) 39(4): 494-501.